Amyloid structures as biofilm matrix scaffolds

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ABSTRACT

Recent insights into bacterial biofilm matrix structures have induced a paradigm shift towards the recognition of amyloid fibers as common building block structures that confer stability to the exopolysaccharidic matrix. Herein, we describe the functional amyloid systems related with biofilm matrix formation in both gram-negative and gram-positive bacteria and recent knowledge regarding the interaction of amyloids with other biofilm matrix components like eDNA and the host immune system. In addition, we summarize the efforts to identify compounds that target amyloid fibers for therapeutic purposes and recent developments that take advantage of the amyloid structure to engineer amyloid fibers of bacterial biofilm matrices for biotechnological applications.

Amyloid structures have been traditionally associated with several incurable degenerative human diseases including Alzheimer's, Parkinson's, Huntington's and prion diseases (1). In these cases, amyloid fibers formation occurs upon aberrant misfolding of soluble and functional proteins (2). However, numerous evidences indicate that amyloid fibers are not just the result of aberrant protein folding, but they are produced on purpose in different non-pathological conditions to provide organisms with beneficial properties (3). The microbial world make use of this type of protein folding for a number of processes relevant for bacterial growth and survival in the environment, such as morphological differentiation of filamentous bacteria (4, 5)), adhesion to host tissues (6), detoxification of toxic compounds (7-9), electron transport (10), and structural scaffolds in biofilm matrices (1).

Amyloid structure is an attractive building material because it is generally resistant to harsh denaturing conditions and in most cases cannot be degraded by proteases (1, 11). In addition, polymerization of amyloid proteins is metabolically cheap because it occurs in the absence of energy. Thus, the amyloid conformation is suitable as a component of the extracellular matrix where the energy sources are limited (12).

The aim of this review is to describe the current knowledge on functional bacterial amyloids involved in building biofilm matrix complexes and highlight the differences on the composition and assembly machineries. Additionally, we will also discuss recently discovered compounds able to destabilize bacterial biofilms through their action on amyloids, and the use of such proteinaceous nanostructures as tools to promote functional biofilms with biotechnological purposes.

Amyloid structures with dedicated fiber assembly machinery

The most studied and characterized bacterial amyloid system is curli fimbriae in

1 *Escherichia coli*. Curli fimbriae show the prototypical biophysical properties of amyloid
2 fibers: ordered β-sheet-rich fibers, resistant to proteases and able to bind amyloid
3 specific dyes (Congo red, Thioflavin-T). Curli assembly requires the products of at least
4 two divergently transcribed operons, *csgBAC* and *csgDEFG* (13). CsgA and CsgB are
5 the protein subunits that make up curli, whereas the rest of the proteins are involved in
6 the regulation of the production, stabilization and secretion of CsgA and CsgB subunits
7 (Figure 1B) (1, 14-16). CsgA has the propensity to self-aggregate, however it requires
8 the CsgB subunit for nucleation into fibers (14, 17). CsgA and CsgB are translocated
9 into the periplasm by the Sec translocon system and exported to the extracellular media
10 through a pore-like structure constituted by the CsgG protein formed in the outer
11 membrane (18). Associated with this pore are the CsgC, CsgE and CsgF proteins, that
12 modulate subunit stability and proper assembly of curli fibers (18, 19). CsgD is the
13 master regulator that controls at transcriptional level the expression of *csgBAC* operon
14 and also *adrA*, involved in post-transcriptional regulation of cellulose synthesis, which
15 is another common component of the biofilm matrix (20-23). CsgD activity is also
16 controlled by a variety of regulatory systems that respond to environmental signals
17 including osmolarity, pH, temperature and nutrient availability (24, 25). Curli fibers
18 mediate bacterial adhesion to a variety of host proteins or abiotic surfaces promoting
19 biofilm formation and pathogenicity (26-31).
20 Production of curli fibers have been described in *Salmonella*, *Citrobacter* and
21 *Enterobacter* (32), but genes with homology to proteins coding for the curli system are
22 found in members of Proteobacteria, Bacteroidetes, Firmicutes and
23 Thermodesulfobacteria (33) suggesting that the formation of curli fibers is a common
24 strategy to provide structural integrity to the biofilm matrix (15).
25 An example of extracellular fibers with structural and functional similarity to curli

1 fimbriae of *E. coli* has been described in various species of *Pseudomonas* including *P.*
2 *aeruginosa*, *P. fluorescens*, and *P. putida* (34). In this case, the protein components for
3 amyloid assembly are encoded in the *fapA-F* operon (35). Biophysical analysis of
4 purified Fap fimbria showed the presence of extensive β-sheet structure, as well as
5 binding to the specific Thioflavin-T dye (35). Biochemical analysis of fibrils
6 composition identified FapC protein as the major protein component, although small
7 amounts of FapB and FapE were also present (35). As for CsgB in curli system, FapB
8 would act as a fibrillation nucleator of FapC (Figure 1B). Fap amyloid fibers facilitate
9 attachment to the abiotic surfaces, and also provide strength to the mature biofilm
10 matrix structure, for instance, in the plant rizosphere (34). Fap fibrils have been shown
11 to bind extracellular metabolites within the biofilm, such as quorum sensing molecules
12 and redox mediators, modulating their release and retention when required. This implies
13 a potential new role for functional amyloids in molecular retention and indirect
14 mediation of cell function (36). Again, genes encoding for proteins with homology to
15 the Fap systems are present within species belonging to the Beta-, Gamma- and
16 Deltaproteobacteria (37).
17 Functional fibers with amyloidogenic properties have been shown to be part of the
18 extracellular matrix of biofilms formed by the soil bacteria *Bacillus subtilis* and *B.*
19 *cereus*. In this case, the biofilm matrix contains TasA made amyloid-like fibers that
20 hold cells together and provide structural support for biofilm maintenance (38, 39).
21 TasA is expressed from the *tapA-sipW-tasA* operon, together with two protein, TapA
22 and SipW, on which depend the formation of the fibrils. The *sipW* gene encodes a type I
23 signal peptidase that is specifically required for the processing and secretion of TasA
24 and TapA (38), whereas TapA is an accessory protein that promotes the efficient
25 polymerization of TasA subunits at the cell envelope (Figure 1A) (9, 40, 41). As well as

for curli, the formation of TasA amyloid fibers is a highly regulated mechanism that involves the participation of a master regulator, SinR, which directly represses the expression of *tapA-sipW-tasA* operon and the biosynthesis of an exopolysaccharide EPS (42). Under conditions favorable for biofilm development, SinI antagonizes the repressive activity of SinR, leading to matrix formation (38). A further level of control of amyloid assembly in *B. subtilis* might be given by the bifunctional signal peptidase, SipW. Disruption of the peptidase active-site prevented the processing of TasA but did not affect biofilm formation capacity, while deletion of the carboxy-terminal region of SipW affected the expression of the repressor SinR, thus impairing the induction of biofilm matrix genes (43).

A special example of extracellular fibers formed by the polymerization of a monomer that it is secreted by a dedicated secretion apparatus corresponds to the phenol soluble modulins (PSMs) in *Staphylococcus aureus* (44). PSMs can be classified according to their length in α-PMSs (20–25 amino acids), and β-PSMs (43–45 amino acids). *S. aureus* expresses four α-PSM peptides encoded in the *αpms* locus, two β-PSM peptides encoded in the *βpsm* locus, and the δ-toxin encoded within RNAIII, the effector molecule of the Agr system {Cheung:2014cz}. These peptides are exported by an ATP-binding cassette (ABC) transporter with two separate membrane components (PmtB and PmtD) and two separate ATPases (PmtA and PmtC) (45). Interestingly, PSM expression is directly regulated by binding of the AgrA response regulator to *psm* loci instead of the effector molecule RNAIII of accessory gene regulator system (Agr) (46). At the extracellular media PSMs can be present either as soluble peptides or as polymerized amyloid structures. When soluble, PSMs form an amphipathic α-helix with surfactant-like properties that promote biofilm disassembly (44, 47). Under still poorly understood environmental conditions, the soluble α-helical peptides switch to β-sheet

rich protein aggregates and in this state, they provide functional support to the biofilm community (Figure 1A) (48). Thus, PSMs are bifunctional proteins that may be stored as inert fibrils in a sessile biofilm until conditions arise that favor their dissociation to promote biofilm disassembly and virulence (44). Recent evidences suggest that PSMs aggregation can be accelerated by the presence of trace amounts of AgrD, the signal peptide of the *S. aureus* quorum-sensing system, since AgrD would self-aggregate and act as seeds, allowing the incorporation of PSMs into the growing aggregate (49).

Surface associated proteins with amyloidogenic properties

Amyloid assembly mechanisms like the ones described above represent sophisticated machineries that strictly control the polymerization of protein subunits into amyloid fibers outside the cell. This is achieved by the expression of accessory genes generally organized within an operon, each of which codifies for a protein with a specific function in amyloid assembly machinery. However, less complex models of auto-aggregative cell-surface proteins with amyloid properties have been also studied in different bacterial genera.

The Bap case is the most recent example of a surface protein with amyloid behavior involved in biofilm development. Bap is a high molecular weight protein present in many staphylococci that is covalently anchored to the peptidoglycan through a sortase-dependent reaction recognizing the LPXTG motif (50, 51). Bap is a multidomain protein structurally organized into four domains. Region A contains two short repeats of 32 amino acids. Region B contains two EF-hand motifs that regulate Bap functionality upon binding to calcium (52). Region C consists of a series of identical repeats of 86 amino acids, followed by a C-terminal region D containing serine-aspartate rich repeated sequence. Because Bap is covalently linked to the cell wall, it was believed

that Bap mediated intercellular adhesion through homophilic interactions between opposing proteins in neighboring cells as it has been shown for SasG protein (53-58). However, recent results indicate that Bap behaves in a completely different manner. After secretion, Bap is proteolytically processed and the N-terminal region released (amino acids 49 to 819) (Figure 1C) *(Taglialegna et al; Plos Pathogens under revision).* The resulting N-terminal fragments adopt a molten globule-like conformation that proceeds to a β-sheet rich form that self-assemble into amyloid-like aggregates when the pH becomes acidic and calcium concentration is below 1mM. At higher calcium concentrations, binding of the cation to the EF-hand domains of region B stabilizes the molten globule-like state hindering the proper self-assembly of the N-terminal region. These findings define a dual function for Bap, first as a sensor and then as a scaffold protein that promotes biofilm development under specific environmental conditions. This mechanism is substantially different to the curli amyloid assembly machinery for several reasons: i) Bap secretion does not require a dedicated secretion machinery because it is efficiently exported when *S. aureus* Newman *bap*-negative strain is complemented with the *bap* gene (59); ii) Bap does not require a chaperon to prevent toxic premature assembly inside the cytoplasm since Bap is assembled extracellularly after protein processing; iii) Bap expression does not require a tightly transcriptional regulation since amyloid formation depends not only on the presence of enough amount of Bap but on specific environmental conditions *(Taglialegna et al; Plos Pathogens under revision).*

Bap is present in coagulase-negative staphylococci and many other bacteria use Bap-like proteins to build a biofilm matrix suggesting that the amyloid-like aggregation described for Bap may be widespread among pathogenic bacteria (51, 59)*(Taglialegna et al; Plos Pathogens under revision)*.

Another example of a surface associated protein with amyloidogenic properties is adhesin P1 (also called antigen I/II) from the cariogenic pathogen *Streptococcus mutans* (60). This protein is found covalently liked to the peptidoglycan and also in the culture media (60). The crystal structure of P1 shows that the protein has a highly extended fibrillar structure in which an alanine-rich repeat region intertwine through an α-helix with the non-contiguous type II helix adopted by the proline-rich repeats region (61). P1 also contains two globular domains, V-region and C-terminus, that assemble into β-sheet–rich fibers characteristics of amyloid fold (Figure 1C) (60, 62, 63).

The α-module of Ag43 autotransporter of *E. coli* constitutes another example of a surface protein with amyloid propensity. Ag43 is composed of two domains: the β-domain, which forms an outer membrane integrated pore and the α-domain, which is secreted to the extracellular environment through the folded β-domain after processing of the Ag43 preprotein. The α and β-domains remain in contact via non covalent interactions (64). Ag43α promotes bacterial auto-aggregation and biofilm formation and it adopts a parallel right-handed β-helix conformation that resemble the amyloid structure established for curli (Figure 1D) (65, 66). However, this structure cannot be considered a typical amyloid fibril because the protein remains anchored to the cell surface and it is unable to polymerize into fibers. Besides, it was described that pairs of α domains mediate interaction between different cells through a "Velcro-like" mechanism in which the formation of α-homodimers organized in a head-to-tail conformation and stabilized by hydrogen bonds and electrostatic interactions, result in the aggregation of cells (64).

Role of biofilm associated extracellular DNA in amyloid polymerization

When studying the role of functional amyloids in the building of an extracellular

bacterial matrix, the often-made question is what actually assists or triggers the assembly of monomeric proteins into fibers, a reaction that is thermodynamically unfavorable and constitutes the rate-limiting step of the reaction (67).

The possibilities to overcome this kinetic limitation range from the presence of trace amounts of preformed aggregates of the same or heterologous protein, binding to other elements present in the cell-envelope or the presence of amyloid prone-to-aggregate stretches into the amino acidic sequence of the protein. As described previously, CsgB, FapB, TapA and AgrD act as fibrillation nucleators. Some recent information pointed out that DNA might also play this role. Direct interaction of DNA with two major bacterial amyloids, curli and PSMs, reveals its role as nucleator of amyloid polymerization (68). In the case of curli fibers, Gallo *et al.* (68) showed that *Salmonella* curli fibers bind tightly to extracellular DNA (eDNA) protecting it from the degradation by DNAses. Furthermore, addition of three different types of nucleic acids (synthetic oligonucleotide, *Salmonella* genomic DNA or eukaryotic DNA) indistinctly reduced the lag phase for curli fibers polymerization, suggesting a mechanism by which DNA molecules trigger the assembly of CsgA monomers into highly stable complexes that would provide stability to *S. typhimurium* biofilms (68).

The presence of eDNA seems to play a key role also in the formation of amyloid fibers in *S. aureus* biofilms. In a recent report, Schwartz *et al.* (69) showed that biofilms formed by *S. aureus* mutant strains, which do not contain eDNA in the extracellular matrix, failed to assemble PSMs amyloid fibrils, even when the monomeric amyloid-like subunits were present (69). Further analysis revealed that DNA was able to promote the polymerization of purified PSMα1 at concentrations in which this peptide is not able to self-polymerize suggesting that DNA increases the peptide concentration above the threshold needed to start fibers assembly by attracting the positively charged PSMs

(69).

Further characterization of the interactions between amyloid fibers and extracellular DNA is necessary to understand how DNA triggers amyloid fibers assembly and how the interaction between these two structures protects the DNA from degradation by DNAses. By extending our current knowledge on the polymerization process and the factors that governs it, the design of molecules or strategies aimed at disrupt amyloid assemblages appears as a wonderful biotechnological tool to fight against biofilms. Examples of first attempts in this direction are provided in further section.

Functional amyloids in microbiome-host interactions.

As a result of life-long commensal permanence of bacteria within the human body, it must be considered that microbial-generated amyloids, together with other related microbial secretory products, could be potential contributors of the pathology of progressive immunological and neurological disorders some of them associated with amyloidogenesis (70). Curli fibers are able to stimulate the innate immune system by recognizing the Toll-like receptor (TLR) 2-TLR1 heterocomplex (71-73). Interestingly, a very recent study highlights the role of curli-DNA complexes in the stimulation of the innate and adaptive immune system and suggests that this complexes can accelerate autoimmunity contributing to Systemic Lupus Erythematosus pathogenesis (68). In an indirect manner, the strong innate immune response activated by bacterial amyloids could stimulate neuropathogenic signals that promote amyloid aggregation and inflammatory degeneration characteristic of age-related neurological diseases such as Alzheimer. Bacterial amyloids leakage through compromised gastrointestinal tract or blood-brain barriers could also directly contribute to progressive neuroamyloidogenesis (70). These data provide evidences of how biofilm-forming bacteria can contribute to

1 the progression of some immunological and neurological diseases and point amyloid
2 components as potential molecular target for their treatment.
3 On the other hand, owing to their immunomodulatory activity, bacterial amyloids have
4 been proposed as potential therapeutic candidates to treat intestinal inflammatory
5 disorders as recently shown by Oppong et al. (74). TLR2 activation by curli fibrils from
6 both commensal and pathogenic microbiota resulted in the production of an
7 immunomodulatory cytokine of intestinal homeostasis that ameliorated inflammation in
8 a mouse model of colitis (74). However, further studies are needed to probe the
9 immunomodulatory function of bacterial amyloids and their use as therapeutic
10 candidates to treat chronic inflammatory disorders.
11
12 **Amyloids as targets to fight against biofilms**
13 Because biofilms can cause devastating consequences in clinical and industrial settings
14 there has been an increasing interest to develop biofilm control strategies. The apparent
15 ubiquity of amyloid fibers in biofilm matrices has led to the screening of molecules that
16 might interrupt its production or assembly.
17 The first molecule to show inhibitory properties against biofilm amyloid fibrils was the
18 ring-fused 2-pyridone called FN075, which is an analogue of BipC10 pilicide
19 compound. FN075 proved to significantly reduce the polymerization of the main curli
20 subunit CsgA and the biogenesis of curli and type 1 pili in uropathogenic *E. coli* (75).
21 This double curlicide-pilicide property of FN075 is important from a therapeutic point
22 of view because *E. coli* can use both structures, pili or curli, to promote surface
23 attachment and subsequent biofilm formation (75). Authors further tested FN075 in a
24 mouse urinary tract infection model to evaluate inhibitory activity *in vivo*. Pretreatment
25 with FN075 of uropathogenic *E. coli* grown under pili-forming but not curli-forming

conditions, significantly attenuated virulence demonstrating the importance of inhibit pili formation to decrease infection (75). However, direct treatment of the mice with the inhibitor to validate its protective role *in vivo* remains to be done.

Romero and collaborators (76) used a simple screening method based on the alteration of *B. subtilis* wrinkly biofilm phenotype to search for possible anti-biofilm compounds. They successfully identified that AA-861, a benzoquinone derivative with anti-inflammatory activity and parthenolide, a sesquiterpene lactone with anti-inflammatory and anti-cancer activities, inhibited TasA polymerization into amyloid-like fibers *in vitro* (76). Interestingly, AA-861 was more efficient to inhibit TasA polymeritization whereas parthenolide was more potent destroying pre-formed biofilms *in vivo* indicating that these molecules can work additively to inhibit biofilm formation (76). Because these two compounds showed anti-biofilm activity against *E. coli* and *B. cereus*, and also impeded the conversion of the yeast New1 protein to its prion state, they seem to have activity against proteins of different origins (76).

Within the field of amyloid fibers and the possible ways to avoid their polymerization, is important to emphasize all the efforts made in fighting against human amyloidogenic degenerative diseases. Several small polyphenol molecules have been demonstrated to remarkably inhibit the formation of fibrillar assemblies *in vitro* and their associated cytotoxicity, being their mechanism primarily based on antioxidative properties or structural constraints (77). The compound (-)-epi-gallocatechine gallate (EGCG), for example, inhibits the fibrillogenesis of α-synuclein and amyloid-β, two amyloid peptides crucially involved in Alzheimer's and Parkinson's diseases respectively, and is a potent remodeling agent of mature amyloid fibrils (78, 79). Although, its role against human amyloid fibrils remains to be demonstrated, the efficiency of EGCG to inhibit *Streptococcus mutans* and *S. aureus* biofilms has been shown (60) *(Taglialegna et al;*

Plos Pathogens under revision). This gives us the clue that compounds able to inhibit human aberrant amyloids could be active also against bacterial functional amyloids, and *vice versa*. In this regard, it has been recently reported that the periplasmic protein CsgC is highly effective inhibiting human α-synuclein and CsgA amyloid formation, while it does not affect polymerization of $A\beta_{42}$ (80).

In a recent report, Rifapentine, an antibiotic that targets RNA polymerase and is often used to treat tuberculosis, was shown to inhibit curli-dependent biofilm formation on different surfaces at a concentration that did not alter bacterial growth (81). Specifically, this antibiotic prevented curli operon transcription as shown by reduction of the levels of mRNA of each *csg* gene in the presence of increasing concentrations of rifapentine. Moreover, expression of cellulose-related genes (*bscA* and *adrA*) that are regulated by CsgD were also decreased in the presence of rifapentine (81).

A mixture of D-amino acids produced by *Bacillus subtilis* displayed the capacity at nanomolar concentration to prevent biofilm formation and disrupt existing biofilms by causing the release of TasA amyloids fibers (82). In the same work it was shown that D-amino acids prevented biofilm formation in *S. aureus* and *P. aeruginosa* (82). However, the effect of D-aminoacids on inhibition of biofilm development must be considered with caution because a recent report was unable to reproduce the inhibitory effect of D-amino acids on biofilm development of *S. aureus* and *B. subtilis* (83).

Finally, the property of protein aggregation and its cytotoxicity can be used to fight against bacterial pathogens during infection (84). Bednarska and collaborators developed an ingenious strategy in this regard: they designed peptides encoding aggregation-prone sequence stretches of bacterial proteins, some of them with an amyloid-like nature, that specifically penetrate bacteria causing aggregation of polypeptides in the cytosol, and ultimately leading to bacterial death. The interesting

point is that these peptides had no detectable effects on host cells (85).

Biotechnological applications of biofilm amyloid fibers

Amyloid fibrils are attractive natural building blocks for the design of new nanostructures, nanomaterials and networks of filaments which can be functionalized for specific applications in nanotechnology and nanomedicine (86). Several applications are being considered for using amyloids as nanowires and nanotubes for electronics, as nanosensors, as amyloid-based gels in cell adhesion and wound healing, and as drug delivery systems (87).

Recently, two elegant studies have utilized the properties of bacterial curli fibers to engineer cellular consortia and obtain artificial biofilms for biotechnological applications. Chen and co-workers (88) developed controllable and autonomous patterning of curli fibrils assembled in *E. coli* cells. The system used external chemical inducers to activate synthetic gene circuits that produced engineered CsgA subunits that self-assemble in functionalized curli fibrils and biofilms. By combining inducer gradients and subunit engineering, this system gives the possibility to fabricate multiscale patterning fibers. The proposed system constitutes a versatile scaffold that is able to synthesize fluorescent quantum dots, gold nanowires, nanorods and nanoparticles, composing switchable conductive biofilms. The other work describes a Biofilm-Integrated Nanofiber Display (BIND) system that consists in genetically program the *E. coli* biofilm extracellular matrix by fusing functional peptide domains to the CsgA protein. This technique is compatible with a wide range of peptide domains of various lengths and secondary structures since they maintain their function after secretion and assembly, conferring artificial functions to the biofilm as a whole (89)(Figure 2B). Based on this work, Botyanszki *et al* (90) exploited the curli system of

E. coli to create a biocatalytic biofilm in which a network of functional nanofibers are capable of covalently and site-specifically immobilize an industrially relevant enzyme, the α -amylase.

So far, curli has been the most engineered bacterial amyloid protein. However, manipulation of others amyloid components present in biofilm matrices of different bacteria might allow the fabrication of functionalized biofilms with different properties. For example, taking advantage of the amyloid assembly properties of Bap, this protein might serve as a potential biotechnological tool by fusing its N-terminal domain to specific peptides tags. The key role of pH and Ca^{2+} in the formation of Bap assemblies could be used to control the disruption or formation of programmed *S. aureus* biofilms (Figure 2A). Because most biofilms in nature are composed by more than one species, it would be interesting to study microbial consortia where multiple engineered amyloidogenic proteins produced by different bacteria could work as a complex nanomaterial to fulfill several technological applications.

Concluding remarks

Functional amyloids are common structural components of the extracellular matrix in bacterial biofilms. In regard to this function, the amyloid conformation provides at least two major advantages compared to other protein mechanisms. Firstly, the assembly of protein monomers into the amyloid insoluble aggregates implies a switch in protein conformation that usually depends on specific environmental conditions. This simple requirement broadens the biofilm regulatory network by providing an additional level of control that enables biofilm development only under particular environmental conditions. Secondly, the stability of the amyloid structure and its intrinsic resistance against proteases prevents the destruction of the biofilm matrix scaffold in the presence

of extracellular proteases. These two obvious advantages may explain why different bacteria coincide in producing proteins with the capacity to form amyloid structures to build the biofilm matrix. However, additional still unknown reasons could justify the use of amyloid fibers as basic building blocks of the bacterial biofilm matrix. Deepening our knowledge in the properties that amyloids provide to biofilm matrix will be fundamental to understand the benefits of living in microbial communities embedded in a self-produced extracellular matrix.

1 **REFERENCE**

2 1. **Blanco LP**, **Evans ML**, **Smith DR**, **Badtke MP**, **Chapman MR**. 2012.
3 Diversity, biogenesis and function of microbial amyloids. Trends Microbiol
4 **20**:66–73.

5 2. **Schwartz K**, **Boles BR**. 2013. Microbial amyloids - functions and interactions
6 within the host. Curr Opin Microbiol **16**:93–99.

7 3. **Otzen D**. 2014. Functional amyloid. Prion **4**:256–264.

8 4. **Sawyer EB**, **Claessen D**, **Haas M**, **Hurgobin B**, **Gras SL**. 2011. The assembly
9 of individual chaplin peptides from *Streptomyces coelicolor* into functional
10 amyloid fibrils. PLoS ONE **6**:e18839.

11 5. **Sawyer EB**, **Claessen D**, **Gras SL**, **Perrett S**. 2012. Exploiting amyloid: how
12 and why bacteria use cross-β fibrils. Biochem Soc Trans **40**:728–734.

13 6. **Alteri CJ**, **Xicohténcatl-Cortes J**, **Hess S**, **Caballero-Olín G**, **Girón JA**,
14 **Friedman RL**. 2007. *Mycobacterium tuberculosis* produces pili during human
15 infection. Proc Natl Acad Sci USA **104**:5145–5150.

16 7. **Shahnawaz M**, **Soto C**. 2012. Microcin amyloid fibrils A are reservoir of toxic
17 oligomeric species. J Biol Chem **287**:11665–11676.

18 8. **Bavdek A**, **Kostanjšek R**, **Antonini V**, **Lakey JH**, **Dalla Serra M**, **Gilbert
19 RJC**, **Anderluh G**. 2012. pH dependence of listeriolysin O aggregation and
20 pore-forming ability. FEBS J **279**:126–141.

21 9. **Romero D**, **Kolter R**. 2014. Functional amyloids in bacteria. Int Microbiol
22 **17**:65–73.

23 10. **Boesen T**, **Nielsen LP**. 2013. Molecular dissection of bacterial nanowires. mBio
24 **4**:e00270–13.

25 11. **Larsen P**, **Nielsen JL**, **Dueholm MS**, **Wetzel R**, **Otzen D**, **Nielsen PH**. 2007.
26 Amyloid adhesins are abundant in natural biofilms. Environ Microbiol **9**:3077–
27 3090.

28 12. **DePas WH**, **Chapman MR**. 2012. Microbial manipulation of the amyloid fold.
29 Res Microbiol **163**:592–606.

30 13. **Hammar M**, **Arnqvist A**, **Bian Z**, **Olsen A**, **Normark S**. 1995. Expression of
31 two csg operons is required for production of fibronectin- and congo red-binding
32 curli polymers in *Escherichia coli* K-12. Mol Microbiol **18**:661–670.

33 14. **Hammer ND**, **McGuffie BA**, **Zhou Y**, **Badtke MP**, **Reinke AA**, **Brännström
34 K**, **Gestwicki JE**, **Olofsson A**, **Almqvist F**, **Chapman MR**. 2012. The C-
35 terminal repeating units of CsgB direct bacterial functional amyloid nucleation. J
36 Mol Biol **422**:376–389.

37 15. **Hobley L**, **Harkins C**, **Macphee CE**, **Stanley-Wall NR**. 2015. Giving structure

1 to the biofilm matrix: an overview of individual strategies and emerging common
2 themes. FEMS Microbiol Rev **39**:649–669.

3 16. **Van Gerven N**, **Klein RD**, **Hultgren SJ**, **Remaut H**. 2015. Bacterial Amyloid
4 Formation: Structural Insights into Curli Biogensis. Trends in Microbiol **23**:693–
5 706.

6 17. **Bian Z**, **Normark S**. 1997. Nucleator function of CsgB for the assembly of
7 adhesive surface organelles in *Escherichia coli.* EMBO J **16**:5827–5836.

8 18. **Robinson LS**, **Ashman EM**, **Hultgren SJ**, **Chapman MR**. 2006. Secretion of
9 curli fibre subunits is mediated by the outer membrane-localized CsgG protein.
10 Mol Microbiol **59**:870–881.

11 19. **Nenninger AA**, **Robinson LS**, **Hammer ND**, **Epstein EA**, **Badtke MP**,
12 **Hultgren SJ**, **Chapman MR**. 2011. CsgE is a curli secretion specificity factor
13 that prevents amyloid fibre aggregation. Mol Microbiol **81**:486–499.

14 20. **Römling U**, **Rohde M**, **Olsen A**, **Normark S**, **Reinköster J**. 2000. AgfD, the
15 checkpoint of multicellular and aggregative behaviour in *Salmonella*
16 *typhimurium* regulates at least two independent pathways. Mol Microbiol **36**:10–
17 23.

18 21. **Simm R**, **Morr M**, **Kader A**, **Nimtz M**, **Römling U**. 2004. GGDEF and EAL
19 domains inversely regulate cyclic di-GMP levels and transition from sessility to
20 motility. Mol Microbiol **53**:1123–1134.

21 22. **Brombacher E**, **Baratto A**, **Dorel C**, **Landini P**. 2006. Gene expression
22 regulation by the Curli activator CsgD protein: modulation of cellulose
23 biosynthesis and control of negative determinants for microbial adhesion. J
24 Bacteriol **188**:2027–2037.

25 23. **Zakikhany K**, **Harrington CR**, **Nimtz M**, **Hinton JCD**, **Römling U**. 2010.
26 Unphosphorylated CsgD controls biofilm formation in Salmonella enterica
27 serovar Typhimurium. Mol Microbiol **77**:771–786.

28 24. **Prigent-Combaret C**, **Brombacher E**, **Vidal O**, **Ambert A**, **Lejeune P**,
29 **Landini P**, **Dorel C**. 2001. Complex regulatory network controls initial adhesion
30 and biofilm formation in *Escherichia coli* via regulation of the *csgD* gene. J
31 Bacteriol **183**:7213–7223.

32 25. **Jubelin G**, **Vianney A**, **Beloin C**, **Ghigo J-M**, **Lazzaroni J-C**, **Lejeune P**,
33 **Dorel C**. 2005. CpxR/OmpR interplay regulates curli gene expression in
34 response to osmolarity in *Escherichia coli*. J Bacteriol **187**:2038–2049.

35 26. **Gophna U**, **Oelschlaeger TA**, **Hacker J**, **Ron EZ**. 2002. Role of fibronectin in
36 curli-mediated internalization. FEMS Microbiol Lett **212**:55–58.

37 27. **Boyer RR**, **Sumner SS**, **Williams RC**, **Pierson MD**, **Popham DL**, **Kniel KE**.
38 2007. Influence of curli expression by *Escherichia coli* 0157:H7 on the cell's
39 overall hydrophobicity, charge, and ability to attach to lettuce. J Food Prot
40 **70**:1339–1345.

1 28. **Pawar DM**, **Rossman ML**, **Chen J**. 2005. Role of curli fimbriae in mediating
2 the cells of enterohaemorrhagic *Escherichia coli* to attach to abiotic surfaces. J
3 Appl Microbiol **99**:418–425.

4 29. **Saldaña Z**, **Xicohténcatl-Cortes J**, **Avelino F**, **Phillips AD**, **Kaper JB**, **Puente**
5 **JL**, **Girón JA**. 2009. Synergistic role of curli and cellulose in cell adherence and
6 biofilm formation of attaching and effacing *Escherichia coli* and identification of
7 Fis as a negative regulator of curli. Environ Microbiol **11**:992–1006.

8 30. **Goulter-Thorsen RM**, **Taran E**, **Gentle IR**, **Gobius KS**, **Dykes GA**. 2011.
9 CsgA production by *Escherichia coli* O157:H7 alters attachment to abiotic
10 surfaces in some growth environments. Appl Environ Microbiol **77**:7339–7344.

11 31. **Zhou Y**, **Smith DR**, **Hufnagel DA**, **Chapman MR**. 2012. Experimental
12 manipulation of the microbial functional amyloid called curli, pp. 53–75. *In*
13 Methods in Molecular Biology. Humana Press, Totowa, NJ.

14 32. **Zogaj X**, **Bokranz W**, **Nimtz M**, **Römling U**. 2003. Production of cellulose and
15 curli fimbriae by members of the family Enterobacteriaceae isolated from the
16 human gastrointestinal tract. Infect Immun **71**:4151–4158.

17 33. **Dueholm MS**, **Albertsen M**, **Otzen D**, **Nielsen PH**. 2012. Curli functional
18 amyloid systems are phylogenetically widespread and display large diversity in
19 operon and protein structure. PLoS ONE **7**:e51274.

20 34. **Dueholm MS**, **Søndergaard MT**, **Nilsson M**, **Christiansen G**, **Stensballe A**,
21 **Overgaard MT**, **Givskov M**, **Tolker-Nielsen T**, **Otzen DE**, **Nielsen PH**. 2013.
22 Expression of Fap amyloids in *Pseudomonas aeruginosa*, *P. fluorescens*, and
23 *P. putida* results in aggregation and increased biofilm formation.
24 Microbiologyopen **2**:365–382.

25 35. **Dueholm MS**, **Petersen SV**, **Sønderkaer M**, **Larsen P**, **Christiansen G**, **Hein**
26 **KL**, **Enghild JJ**, **Nielsen JL**, **Nielsen KL**, **Nielsen PH**, **Otzen DE**. 2010.
27 Functional amyloid in Pseudomonas. Mol Microbiol **77**:1009–1020.

28 36. **Seviour T**, **Hansen SH**, **Yang L**, **Yau YH**, **Wang VB**, **Stenvang MR**,
29 **Christiansen G**, **Marsili E**, **Givskov M**, **Chen Y**, **Otzen DE**, **Nielsen PH**,
30 **Geifman-Shochat S**, **Kjelleberg S**, **Dueholm MS**. 2015. Functional amyloids
31 keep quorum-sensing molecules in check. J Biol Chem **290**:6457–6469.

32 37. **Dueholm MS**, **Otzen D**, **Nielsen PH**. 2013. Evolutionary insight into the
33 functional amyloids of the pseudomonads. PLoS ONE **8**:e76630.

34 38. **Branda SS**, **Chu F**, **Kearns DB**, **Losick R**, **Kolter R**. 2006. A major protein
35 component of the *Bacillus subtilis* biofilm matrix. Mol Microbiol **59**:1229–1238.

36 39. **Romero D**, **Aguilar C**, **Losick R**, **Kolter R**. 2010. Amyloid fibers provide
37 structural integrity to *Bacillus subtilis* biofilms. Proc Natl Acad Sci USA
38 **107**:2230–2234.

39 40. **Romero D**, **Vlamakis H**, **Losick R**, **Kolter R**. 2011. An accessory protein
40 required for anchoring and assembly of amyloid fibres in *B. subtilis* biofilms.

1 Mol Microbiol **80**:1155–1168.

2 41. **Romero D**, **Vlamakis H**, **Losick R**, **Kolter R**. 2014. Functional analysis of the
3 accessory protein TapA in *Bacillus subtilis* amyloid fiber assembly. J Bacteriol
4 **196**:1505–1513.

5 42. **Kearns DB**, **Chu F**, **Branda SS**, **Kolter R**, **Losick R**. 2005. A master regulator
6 for biofilm formation by *Bacillus subtilis*. Mol Microbiol **55**:739–749.

7 43. **Terra R**, **Stanley-Wall NR**, **Cao G**, **Lazazzera BA**. 2012. Identification of
8 *Bacillus subtilis* SipW as a bifunctional signal peptidase that controls surface-
9 adhered biofilm formation. J Bacteriol **194**:2781–2790.

10 44. **Schwartz K**, **Syed AK**, **Stephenson RE**, **Rickard AH**, **Boles BR**. 2012.
11 Functional amyloids composed of phenol soluble modulins stabilize
12 *Staphylococcus aureus* biofilms. PLoS Pathogens **8**:e1002744.

13 45. **Chatterjee SS**, **Joo H-S**, **Duong AC**, **Dieringer TD**, **Tan VY**, **Song Y**, **Fischer**
14 **ER**, **Cheung GYC**, **Li M**, **Otto M**. 2013. Essential Staphylococcus aureus toxin
15 export system. Nat Med **19**:364–367.

16 46. **Queck SY**, **Jameson-Lee M**, **Villaruz AE**, **Bach T-HL**, **Khan BA**, **Sturdevant**
17 **DE**, **Ricklefs SM**, **Li M**, **Otto M**. 2008. RNAIII-independent target gene control
18 by the agr quorum-sensing system: insight into the evolution of virulence
19 regulation in *Staphylococcus aureus*. Molecular Cell **32**:150–158.

20 47. **Vuong C**, **Götz F**, **Otto M**. 2000. Construction and characterization of an agr
21 deletion mutant of *Staphylococcus epidermidis*. Infec Immun **68**:1048–1053.

22 48. **Syed AK**, **Boles BR**. 2014. Fold modulating function: bacterial toxins to
23 functional amyloids. Front Microbiol **5**:401.

24 49. **Schwartz K**, **Sekedat MD**, **Syed AK**, **O'Hara B**, **Payne DE**, **Lamb A**, **Boles**
25 **BR**. 2014. The AgrD N-Terminal leader peptide of *Staphylococcus aureus* has
26 cytolytic and amyloidogenic properties. Infect Immun **82**:3837–3844.

27 50. **Cucarella C**, **Solano C**, **Valle J**, **Amorena B**, **Lasa I**, **Penadés JR**. 2001. Bap, a
28 *Staphylococcus aureus* surface protein involved in biofilm formation. J Bacteriol
29 **183**:2888–2896.

30 51. **Tormo MA**, **Knecht E**, **Götz F**, **Lasa I**, **Penadés JR**. 2005. Bap-dependent
31 biofilm formation by pathogenic species of *Staphylococcus*: evidence of
32 horizontal gene transfer? Microbiology (Reading, Engl) **151**:2465–2475.

33 52. **Arrizubieta MJ**, **Toledo-Arana A**, **Amorena B**, **Penadés JR**, **Lasa I**. 2004.
34 Calcium inhibits bap-dependent multicellular behavior in *Staphylococcus aureus*.
35 J Bacteriol **186**:7490–7498.

36 53. **Geoghegan JA**, **Corrigan RM**, **Gruszka DT**, **Speziale P**, **O'Gara JP**, **Potts JR**,
37 **Foster TJ**. 2010. Role of surface protein SasG in biofilm formation by
38 *Staphylococcus aureus*. J Bacteriol **192**:5663–5673.

1 54. **Barbu EM**, **Mackenzie C**, **Foster TJ**, **Höök M**. 2014. SdrC induces
2 staphylococcal biofilm formation through a homophilic interaction. Mol
3 Microbiol **94**:172–185.

4 55. **Missineo A**, **Di Poto A**, **Geoghegan JA**, **Rindi S**, **Heilbronner S**, **Gianotti V**,
5 **Arciola CR**, **Foster TJ**, **Speziale P**, **Pietrocola G**. 2014. IsdC from
6 *Staphylococcus lugdunensis* induces biofilm formation under low-iron growth
7 conditions. Infect Immun **82**:2448–2459.

8 56. **Conrady DG**, **Brescia CC**, **Horii K**, **Weiss AA**, **Hassett DJ**, **Herr AB**. 2008. A
9 zinc-dependent adhesion module is responsible for intercellular adhesion in
10 staphylococcal biofilms. Proc Natl Acad Sci USA **105**:19456–19461.

11 57. **Herman-Bausier P**, **El-Kirat-Chatel S**, **Foster TJ**, **Geoghegan JA**, **Dufrêne**
12 **YF**. 2015. *Staphylococcus aureus* Fibronectin-binding protein A mediates cell-
13 cell adhesion through low-affinity homophilic bonds. mBio **6**:e00413–15.

14 58. **Formosa-Dague C**, **Speziale P**, **Foster TJ**, **Geoghegan JA**, **Dufrêne YF**. 2015.
15 Zinc-dependent mechanical properties of Staphylococcus aureus biofilm-forming
16 surface protein SasG. Proceedings of the National Academy of Sciences.

17 59. **Lasa I**, **Penadés JR**. 2006. Bap: a family of surface proteins involved in biofilm
18 formation. Res Microbiol **157**:99–107.

19 60. **Oli MW**, **Otoo HN**, **Crowley PJ**, **Heim KP**, **Nascimento MM**, **Ramsook CB**,
20 **Lipke PN**, **Brady LJ**. 2012. Functional amyloid formation by *Streptococcus*
21 *mutans.* Microbiology **158**:2903–2916.

22 61. **Larson MR**, **Rajashankar KR**, **Patel MH**, **Robinette RA**, **Crowley PJ**,
23 **Michalek S**, **Brady LJ**, **Deivanayagam C**. 2010. Elongated fibrillar structure of
24 a streptococcal adhesin assembled by the high-affinity association of alpha- and
25 PPII-helices. Proc Natl Acad Sci USA **107**:5983–5988.

26 62. **Larson MR**, **Rajashankar KR**, **Crowley PJ**, **Kelly C**, **Mitchell TJ**, **Brady LJ**,
27 **Deivanayagam C**. 2011. Crystal structure of the C-terminal region of
28 Streptococcus mutans antigen I/II and characterization of salivary agglutinin
29 adherence domains. J Biol Chem **286**:21657–21666.

30 63. **Troffer-Charlier N**, **Ogier J**, **Moras D**, **Cavarelli J**. 2002. Crystal structure of
31 the V-region of *Streptococcus mutans* antigen I/II at 2.4 A resolution suggests a
32 sugar preformed binding site. J Mol Biol **318**:179–188.

33 64. **Heras B**, **Totsika M**, **Peters KM**, **Paxman JJ**, **Gee CL**, **Jarrott RJ**, **Perugini**
34 **MA**, **Whitten AE**, **Schembri MA**. 2013. The antigen 43 structure reveals a
35 molecular Velcro-like mechanism of autotransporter-mediated bacterial clumping.
36 Proc Natl Acad Sci USA 1–6.

37 65. **Klemm P**, **Hjerrild L**, **Gjermansen M**, **Schembri MA**. 2004. Structure-
38 function analysis of the self-recognizing Antigen 43 autotransporter protein from
39 Escherichia coli. Mol Microbiol **51**:283–296.

40 66. **Knudsen SK**, **Stensballe A**, **Franzmann M**, **Westergaard UB**, **Otzen DE**.

2008. Effect of glycosylation on the extracellular domain of the Ag43 bacterial autotransporter: enhanced stability and reduced cellular aggregation. Biochem J **412**:563–577.

67. **Buell AK**, **Dobson CM**, **Knowles TPJ**. 2014. The physical chemistry of the amyloid phenomenon: thermodynamics and kinetics of filamentous protein aggregation. Essays Biochem **56**:11–39.

68. **Gallo PM**, **Rapsinski GJ**, **Wilson RP**, **Oppong GO**, **Sriram U**, **Goulian M**, **Buttaro B**, **Caricchio R**, **Gallucci S**, **Tükel C**. 2015. Amyloid-DNA Composites of Bacterial Biofilms Stimulate Autoimmunity. Immunity **42**:1171–1184.

69. **Schwartz K**, **Ganesan M**, **Payne DE**, **Solomon MJ**, **Boles BR**. 2016. Extracellular DNA facilitates the formation of functional amyloids in *Staphylococcus aureus* biofilms. Mol Microbiol **99**:123–134.

70. **Zhao Y**, **Lukiw WJ**. 2015. Microbiome-generated amyloid and potential impact on amyloidogenesis in Alzheimer's disease (AD). J Nat Sci **1**.

71. **Tükel C**, **Raffatellu M**, **Humphries AD**, **Wilson RP**, **Andrews-Polymenis HL**, **Gull T**, **Figueiredo JF**, **Wong MH**, **Michelsen KS**, **Akçelik M**, **Adams LG**, **Bäumler AJ**. 2005. CsgA is a pathogen-associated molecular pattern of Salmonella enterica serotype Typhimurium that is recognized by Toll-like receptor 2. Mol Microbiol **58**:289–304.

72. **Tükel C**, **Wilson RP**, **Nishimori JH**, **Pezeshki M**, **Chromy BA**, **Bäumler AJ**. 2009. Responses to amyloids of microbial and host origin are mediated through toll-like receptor 2. Cell Host & Microbe **6**:45–53.

73. **Tükel C**, **Nishimori JH**, **Wilson RP**, **Winter MG**, **Keestra AM**, **van Putten JPM**, **Bäumler AJ**. 2010. Toll-like receptors 1 and 2 cooperatively mediate immune responses to curli, a common amyloid from enterobacterial biofilms. Cell Microbiol **12**:1495–1505.

74. **Oppong GO**, **Rapsinski GJ**, **Tursi SA**, **Biesecker SG**, **Klein-Szanto AJ**, **Goulian M**, **McCauley C**, **Healy C**, **Tükel RPWAC**. 2015. Biofilm-associated bacterial amyloids dampen inflammationin the gut: oral treatment with curli fibres reduces theseverity of hapten-induced colitis in mice. Biofilms and Microbiomes 1–8.

75. **Cegelski L**, **Pinkner JS**, **Hammer ND**, **Cusumano CK**, **Hung CS**, **Chorell E**, **Aberg V**, **Walker JN**, **Seed PC**, **Almqvist F**, **Chapman MR**, **Hultgren SJ**. 2009. Small-molecule inhibitors target *Escherichia coli* amyloid biogenesis and biofilm formation. Nat Chem Biol **5**:913–919.

76. **Romero D**, **Sanabria-Valentín E**, **Vlamakis H**, **Kolter R**. 2013. Biofilm inhibitors that target amyloid proteins. Chem Biol **20**:102–110.

77. **Porat Y**, **Abramowitz A**, **Gazit E**. 2006. Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. Chem Biol Drug Des **67**:27–37.

1 78. **Bieschke J**, **Russ J**, **Friedrich RP**, **Ehrnhoefer DE**, **Wobst H**, **Neugebauer K**,
2 **Wanker EE**. 2010. EGCG remodels mature alpha-synuclein and amyloid-beta
3 fibrils and reduces cellular toxicity. Proc Natl Acad Sci USA **107**:7710–7715.

4 79. **Ehrnhoefer DE**, **Bieschke J**, **Boeddrich A**, **Herbst M**, **Masino L**, **Lurz R**,
5 **Engemann S**, **Pastore A**, **Wanker EE**. 2008. EGCG redirects amyloidogenic
6 polypeptides into unstructured, off-pathway oligomers. Nat Struct Mol Biol
7 **15**:558–566.

8 80. **Evans ML**, **Chorell E**, **Taylor JD**, **Åden J**, **Götheson A**, **Li F**, **Koch M**, **Sefer**
9 **L**, **Matthews SJ**, **Wittung-Stafshede P**, **Almqvist F**, **Chapman MR**. 2015. The
10 bacterial curli system possesses a potent and selective inhibitor of amyloid
11 formation. Molecular Cell **57**:445–455.

12 81. **Maher MC**, **Lim JY**, **Gunawan C**, **Cegelski L**. 2015. Cell-Based High-
13 Throughput Screening Identifies Rifapentine. ACS Infect Dis 150908134623006.

14 82. **Kolodkin-Gal I**, **Romero D**, **Cao S**, **Clardy J**, **Kolter R**, **Losick R**. 2010. D-
15 amino acids trigger biofilm disassembly. Science **328**:627–629.

16 83. **Sarkar S**, **Pires MM**. 2015. D-Amino acids do not inhibit biofilm formation in
17 *Staphylococcus aureus*. PLoS ONE **10**:e0117613.

18 84. **Ventura S**. 2015. Curing bacterial infections with protein aggregates. Mol
19 Microbiol. 10.1111/mmi.13293

20 85. **Bednarska NG**, **Van Eldere J**, **Gallardo R**, **Ganesan A**, **Ramakers M**, **Vogel I**,
21 **Baatsen P**, **Staes A**, **Goethals M**, **Hammarström P**, **Nilsson KPR**, **Gevaert K**,
22 **Schymkowitz J**, **Rousseau F**. 2015. Protein aggregation as an antibiotic design
23 strategy. Mol Microbiol. 10.1111/mmi.13269

24 86. **Cherny I**, **Gazit E**. 2008. Amyloids: not only pathological agents but also
25 ordered nanomaterials. Angew Chem Int Ed Engl **47**:4062–4069.

26 87. **Hauser CAE**, **Maurer-Stroh S**, **Martins IC**. 2014. Amyloid-based nanosensors
27 and nanodevices. Chem Soc Rev **43**:5326.

28 88. **Chen AY**, **Deng Z**, **Billings AN**, **Seker UOS**, **Lu MY**, **Citorik RJ**, **Zakeri B**,
29 **Lu TK**. 2014. Synthesis and patterning of tunable multiscale materials with
30 engineered cells. Nat Mater **13**:515–523.

31 89. **Nguyen PQ**, **Botyanszki Z**, **Tay PKR**, **Joshi NS**. 2014. Programmable biofilm-
32 based materials from engineered curli nanofibres. Nat Commun **5**:4945.

33 90. **Botyanszki Z**, **Tay PKR**, **Nguyen PQ**, **Nussbaumer MG**, **Joshi NS**. 2015.
34 Engineered catalytic biofilms: Site-specific enzyme immobilization onto E. coli
35 curli nanofibers. Biotechnol Bioeng **112**:2016–2024.

36

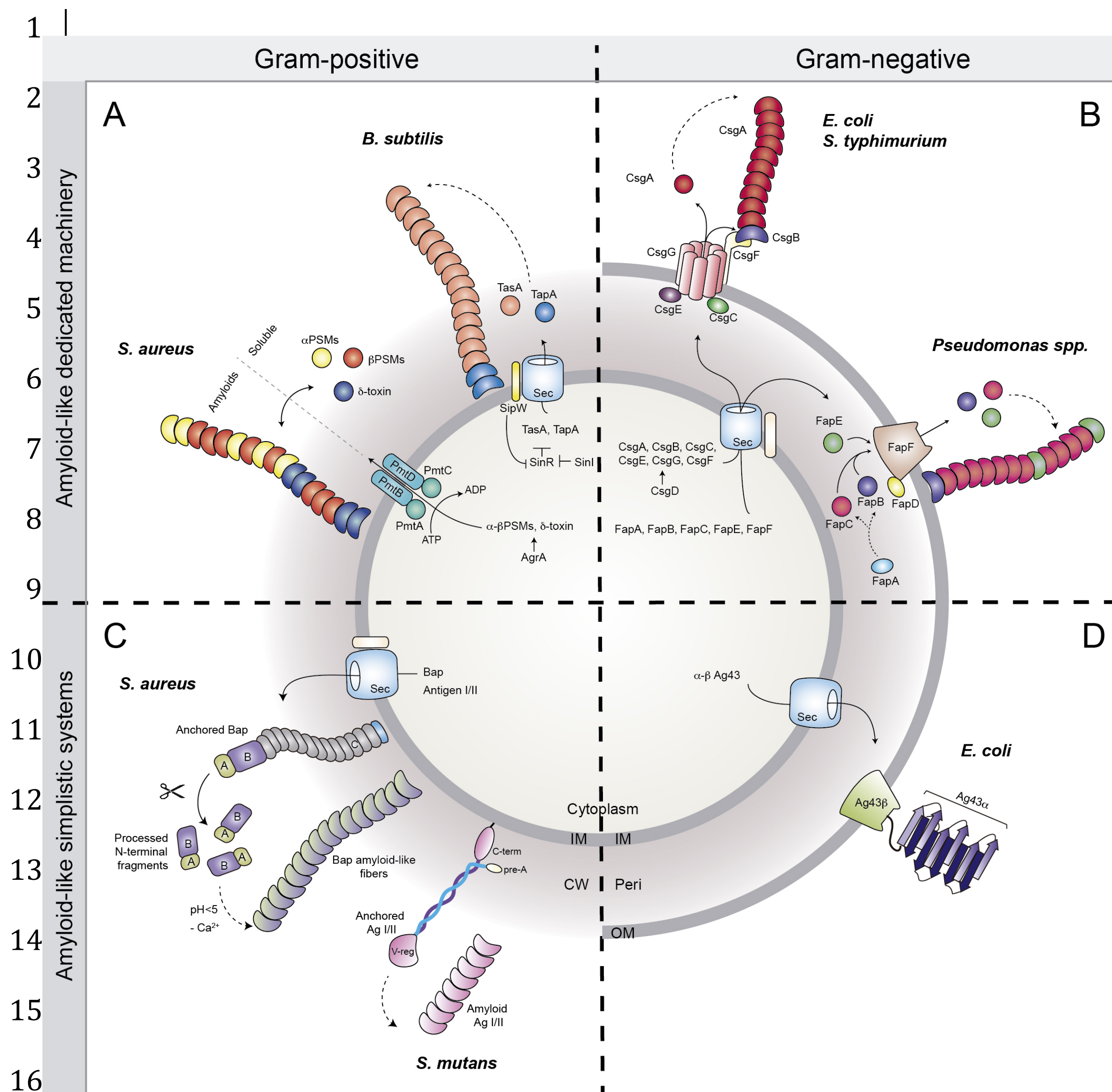

Figure 1. Dedicated or simplistic amyloid-like fibers formation systems in gram-positive and gram-negative bacteria. A) Sec export system secretes the products of *tapA-sipW-tasA* operon through the inner membrane (IM) of *B. subtilis*. SipW acts as a signal peptidase cleaving the signal peptide from TasA and TapA. TapA allows the anchorage of fibers to the cell wall (CW), and TasA is the major component of the fibers. SinR represses the operon, but when conditions are propitious for biofilm formation SinI antagonizes SinR. The C-terminus of SipW has also a regulatory effect

1 on the operon. In *S. aureus* αPSM, βPSMs and δ-toxin monomers are transported
2 outside the cell through an ATP-dependent ABC transporter (PmtB and PmtD
3 transmembrane proteins, linked to ATPases PmtA and PmtC). PSMs can be present as
4 soluble monomers, or, when required, forming amyloid-like fibers. Expression of α and
5 β *psm* genes is induced by AgrA, the response regulator of the Agr system. **B)** All Fap
6 and Csg proteins (except for CsgD) are expulsed via Sec across the inner membrane. In
7 the case of curli, the major fiber subunit CsgA and the nucleator CsgB are secreted
8 across the outer membrane (OM) through CsgG channel, and once on the cell surface
9 they start to polymerize into amyloidogenic fibers. Accesory proteins CsgC and CsgE
10 regulate export by CsgG, and CsgF assist the nucleation of CsgA. CsgD is the master
11 regulator of *csgBAC* operon. In *Pseudomonas,* Fap B, FapC and FapE are further
12 secreted across the outer membrane through FapF where FapB nucleates fiber assembly
13 of FapC and, in minor proportion, FapE. FapA likely control FapB and FapC secretion,
14 and FapD would act as a protease of Fap proteins. **C)** Once is anchored to the cell wall,
15 Bap of *S. aureus* is processed by proteases present in the medium, liberating the N-
16 terminal fragments that will ultimately self-assemble into amyloid like fibrillar
17 structures upon acidification of the media and in the absence of calcium. In *S. mutans,*
18 non-attached P1 adhesin (AgI/II) would presumably form amyloidogenic fibers through
19 its β-sheet rich V-region and C-terminal domain. **D)** The autotransporter Ag43 present
20 in *E. coli*, though not considered a real amyloid, represent a very simplistic model of
21 amyloid folding due to the high β-helix with β-strand conformation adopted by Ag43α
22 subunit at the bacterial envelope.
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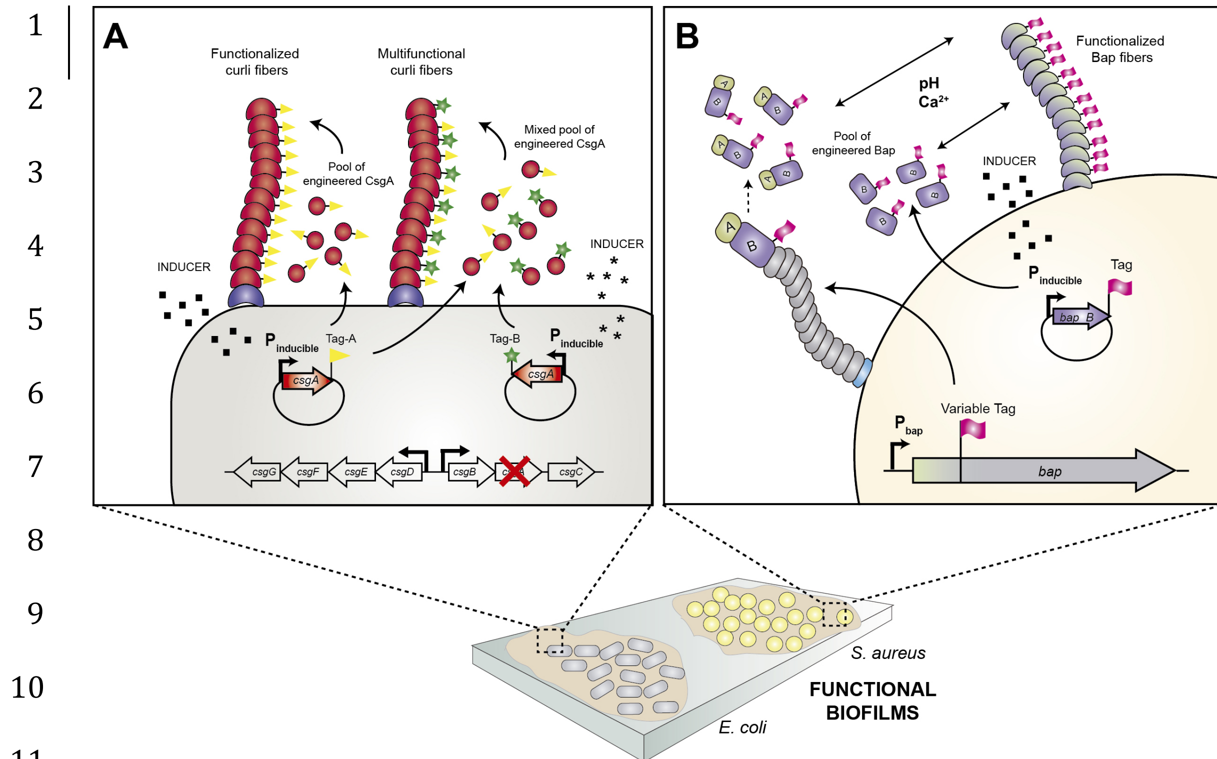

Figure 2. Development of functionalized amyloid-dependent biofilms in gram-negative and gram-positive bacteria. A) In *E. coli*, the major curli protein CsgA is engineered by fusing variable tags at its C-terminus. Once in the extracellular media, the fusion protein self-assembles into functional amyloid nanofibers. It is possible to obtain multifunctional biofilms by expressing engineered CsgA proteins under the control of different inducible promoters. The addition of measured concentrations of inducer molecules grants the expression and production of curli fibers containing precise multiple designed functions as a result of random extracellular self-assembly of CsgA monomers. **B)** In the case of *S. aureus*, variable tags fused to the C-terminal part of B domain of Bap could allow the formation of engineered Bap fibers. Since B domain of Bap (amino acids 362 to 819) is sufficient to bestow multicellular behavior, it could be possible to express engineered Bap_B proteins under the control of an inducible promoter. The property of Bap to reversibly form amyloids according to pH and Ca^{2+}

1 levels in the media could be used as an external way to control the formation or

2 disruption of functionalized biofilms.

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2 **Table 1. Summary of compounds showing suppressive effect on bacterial amyloids.**

Amyloid inhibitors	Anti-biofilm effect	Bacterial amyloid target	Eukaryotic amyloid target	Reference
FN075	*E. coli*	Curli Type-I pili		(75)
Rifapentine	*E. coli*	Curli		(81)
D-aminoacids	*B. subtilis* *S. aureus* *Pseudomonas*	TasA		(82)
AA-861	*B. subtilis* *B. cereus* *E. coli*	TsaA Curli	Yeast New1	(76)
Parthenolide	*B. subtilis* *B. cereus* *E. coli*	TsaA Curli	Yeast New1	(76)
EGCG	*S. mutants* *S. aureus*	P1 adhesin Bap	α -synuclein Amiloid-β	(60) Taglialegna et al
CsgC	-	Curli	α-synuclein	(80)

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