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# Bacterial Functional Amyloids: Order from Disorder

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## Abstract

The discovery of intrinsic disorderness in proteins and peptide regions has given a new and useful insight into the working of biological systems. Due to enormous plasticity and heterogeneity, intrinsically disordered proteins or regions in proteins can perform myriad of functions. The flexibility in disordered proteins allows them to undergo conformation transition to form homopolymers of proteins called amyloids. Amyloids are highly structured protein aggregates associated with many neurodegenerative diseases. However, amyloids have gained much appreciation in recent years due to their functional roles. A functional amyloid fiber called curli is assembled on the bacterial cell surface as a part of the extracellular matrix during biofilm formation. The extracellular matrix that encases cells in a biofilm protects the cells and provides resistance against many environmental stresses. Several of the Csg (**c**urli **s**pecific **g**enes) proteins that are required for curli amyloid assembly are predicted to be intrinsically disordered. Therefore, curli amyloid formation is highly orchestrated so that these intrinsically disordered proteins do not inappropriately aggregate at the wrong time or place. The curli proteins are compartmentalized and there are chaperone-like proteins that prevent inappropriate aggregation and allow the controlled assembly of curli amyloids. Here we review the biogenesis of curli amyloids and the role that intrinsically disordered proteins play in the process.

### Keywords

Intrinsically disordered proteins; phase transition; functional amyloids; curli; biofilms

## 1. The dynamics of unstructured proteins

Until recently, there was an assumption that protein function must be tightly linked to a definable globular structure [1]. Hence, the “sequence-structure-function paradigm” that

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posits the globular structure is determined by the amino acid sequence, which in turn dictates the function of the protein, is well-conceived and accepted [1]. However, the advent of new technologies and discoveries has introduced the idea that some proteins are functional in a 'natively disordered' state [2]. Natively disordered or **i**ntrinsically **d**isordered **p**roteins (IDPs) are classically defined as proteins that lack a stable definitive globular structure [3]. IDPs exist in a dynamic ensemble of conformations that enables them to functionally engage with multiple substrates [4]. Some proteins have discrete domains or sequences that are intrinsically disordered, called IDRs (**i**ntrinsically **d**isordered **r**egions) [5]. It is now well established that IDPs and IDRs contribute to various cellular biological processes [4].

Pontius and Berg were the first to link protein disorder and function when they identified a functional IDR in the C-terminus of hnRNP A1[6]. Pontius proposed that IDRs may have higher utility since they can switch the conformations rapidly and can bind to multiple partners [6]. Later, bioinformatic analyses found that 'disorderness' varies among proteins, but can be classified as the following: 1) 'molecular switches' that fold upon binding, 2) 'morphers' that vary in structure based on their partners, 3) 'dormant disorder' where disorder is triggered by interacting with other proteins or molecules, and 4) 'fuzzies' that remain flexible even after binding [3]. The hypothesis that particular amino acid sequences beget disorder was tested by developing predictors to predict fold based on amino acid sequences [7]. The results from these predictors suggested that peptide chains with less hydrophobic and more charged amino acids are likely to remain unfolded or disordered[8]. IDPs are typically rich in amino acids like M, K, R,S, Q, P, E and significantly depleted in W, Y, F, V, L, C [9]. Therefore, combinations of certain amino acids allow IDPs to remain in a dynamically unfolded state [4][10].

The astonishing dynamics possessed by IDPs gives them the ability to switch between structures in a very short time scale [9]. It is a challenge to structurally characterize IDPs using conventional biochemical and biophysical techniques like X-ray crystallography [11]. Web based prediction software like PONDR, DISOPRED, GlobPlot, and FoldIndex have been successful in identifying the propensity of intrinsic disorderness in a protein sequence [12]. Bioinformatic tools like molecular dynamic (MD) simulations (CAMPRI and Rosetta) have been crucial to understand the structural dynamics of IDPs [13]. Nuclear magnetic resonance (NMR) has emerged as a powerful probe that has been widely and successfully used to map flexibility, dynamics and binding mechanism in IDPs under both *in vitro* and cell based environments [14]. New and more sophisticated techniques like single molecule atomic force microscopy and single molecule FRET (fluorescence resonance energy transfer) have been recently implemented to understand the conformational polymorphism in IDPs [15].

## 2. Role of IDPs in phase transition

Owing to low complexity and high net charge IDPs and IDRs are most suitable to undergo liquid-liquid phase transition to confine certain biological molecules and reactions in compartments within nucleus or cytoplasm and therefore making proteinaceous membrane less organelles (PMLO) [16,17]. PMLOs are formed in response to environmental changes

and are the result of regulated liquid-liquid phase transition [16,17] which is driven by the primary sequence of the protein components [18-20]. The current knowledge on protein component in PMLO is limited. However, recent studies indicate that the presence of IDPs or IDRs may be a prerequisite for formation of formation of dynamic, cell-size dependent PMLOs [16].The highly dynamic nature of PMLOs allows them to participate in a wide range of molecular interactions [21]. A recent review by *Darling et al* provides a comprehensive list of IDPs and IDRs involved in PMLO formation [21]. The review also highlighted presence of disordered proteins linked to PMLOs in different organisms suggesting IDPs/IDRs to be an evolutionary conserved factor [21]. IDPs are also involved in phase transition and formation of membrane-less organelles in plants [22]. Finally, IDPs that phase transition can drive the formation of structured homoaggregates or amyloids associated with neurodegenerative diseases [23-26].

## 3. IDPs and amyloid formation (disease related and functional)

The conformational dynamics of IDPs may act as double edged sword where it allows the polypeptide chain to perform various functions but is also poised to collapse to form “molten globules” that lead to oligomer and amyloid formation [25]. Amyloids are highly ordered cross β-sheet structures of proteins that are commonly associated with neurodegenerative disorders like Alzheimer’s, Parkinson’s and Huntington’s disease [27]. Some natively folded, globular proteins become denatured prior to forming amyloid [28]. However, owing to intrinsic disorderedness, IDPs are prone to amyloid aggregation. α-Synuclein (α-syn) is one such IDP that has a high level of structural plasticity and undergoes conformation transitions upon binding to different substrates [29]. Amyloid formation by α-synuclein is linked with pathophysiology of Parkinson’s disease [30]. As illustrated in figure 1, disordered α-synuclein undergoes membrane-induced conformational switch to helical structure. The membrane bound helical α-synuclein has higher propensity to aggregate and form β-sheet rich amyloids [31].

Although readily recognized as the product of protein misfolding and aggregation, some amyloids are beneficial for cells [32]. These so called ‘functional’ amyloids can be found in all walks of life from bacteria to mammals [33]. Functional amyloids made by bacteria are an important constituent of the extracellular matrix that protects cells from environmental stressors [34]. Cytoplasmic Polyadenylation Element Binding (CPEB) from sea snails, and its homolog in Drosophila, are functional amyloids that help with memory consolidation [35,36]. Humans and other mammals possess homologs of CPEB that are putatively involved in similar functions [37]. One of the fascinating ways of utilizing amyloid scaffold is to store peptide hormones in exocrine and endocrine glands. Upon receiving appropriate signals, the amyloid form of hormones gets disaggregate to release the peptides [38].

The subunit proteins that make up functional amyloid fibers are often intrinsically disordered, thus making the monomers prone to oligomerization and aggregation [39]. Therefore, the biogenesis of functional amyloids must be spatially and temporarily regulated such that potential cellular toxicity is mitigated [40]. Certain functional amyloids transition from monomer to amyloid polymer efficiently enough to limit the accumulation of oligomeric intermediates [41]. For instance, the human functional amyloid Pmel forms

ordered amyloid aggregates at a much faster rate compared to disease-associated proteins like Aβ and α-synuclein [42]. Other functional amyloids have adapted 'nucleator' proteins that provide an amyloid-like scaffold which can be used as a folding template to accelerate amyloid formation [43]. Dedicated chaperone proteins can also prevent amyloid formation at the wrong time or the wrong place within the cell [44-47].

For the remainder of the review we will discuss the functional amyloid systems with a focus on the bacterial amyloid called curli. The curli amyloid assembly system provides many elegant examples of how cells can control disordered and highly aggregation prone proteins.

## 4. Curli proteins in *Escherichia coli:* the disordered form

Bacteria are generally found in sessile communities called biofilms, where individual cells can be adhered to other cells or to a surface by biopolymers assembled on the cell surface [48]. Since the extracellular space will be exposed to environmental stresses, it is not surprising that the protein polymers in the extracellular matrix (ECM) are often amyloids [34]. The functional amyloid fiber (called curli) found in the extracellular matrix of *E. coli* are heteropolymers of two proteins, CsgA and CsgB [49].CsgA and CsgB are IDPs with flexible and aggregation prone regions [49]. In a test tube, purified CsgA and CsgB exist transiently in a random coil conformation, but then aggregate into ordered amyloid fibers [50].Inside the cell an efficient chaperone network helps to discourage CsgA and CsgB aggregation, so that CsgA and CsgB can be secreted out of the cell where they polymerize into curli amyloid fibers [44,46]. The *csgA* and *csgB* genes chaperoned and secreted to the cell surface with the help of proteins encoded on a divergently transcribed operon, *csgDEFG* [34]. Figure 2A illustrates how Csg proteins coordinate to assemble curli amyloid fibers on the bacterial cell surface. The next sections are dedicated to the biogenesis of curli on cell surface and the regulatory machinery that keeps a check on intracellular aggregation.

### 4.1 The major curli subunit; CsgA:

The major curli subunit CsgA, is secreted to the cell surface as a soluble disordered polypeptide chain where it efficiently adopts a cross-β-strand polymer conformation that is the hallmark of all amyloids [43]. CsgA polypeptide contains an N-terminal 22 amino acid stretch that is required for translocation through the outer membrane secretion complex [51]. CsgA is capable of adopting the distinctive amyloid conformation under different *in vitro* conditions [52,53]. As shown in figure 2B, the transition of soluble disordered CsgA monomer to mature fibrils is a nucleation-dependent process and involves formation of transient intermediates [54]. Recent high resolution studies suggests that CsgA fiber formation is a one-step nucleation process wherein CsgA monomers fold and oligomerize to form a minimum fiber unit that contains all the characteristic features of mature fibers [55]. CsgA amyloid polymerization can also be accelerated or 'seeded' by the addition of sonicated preformed fibrils, which significantly decreases the lag phase [54]. Interestingly, *E. coli* CsgA exhibits seeding promiscuity and its amyloid formation can be accelerated by CsgA homologs from *Citrobacter koseri* and *Salmonella typhimurium* [56].

Due to presence of conserved amino acids, the CsgA amyloid core can be divided into five imperfect repeat regions (R1-R5) [39]. Each of the CsgA repeats contains a Ser-$X_5$-Gln-$X_4$-

Asn-$X_5$-Gln motif that appears conserved in all CsgA homologs from *Enterobacteriaceae* [54,57]. In-depth mutagenesis analysis revealed that the conserved Gln (glutamine) and Asn (asparagine) residues in the repeat regions at N-and C-terminus play an essential role in CsgB mediated curli formation *in vivo* and CsgA self-assembly *in vitro* [50]. More specifically, Gln residues at position 49 and 139 and Asn residues at 54 and 144 position were found to be indispensable for side chain interactions and curli assembly since mutating these residues renders no curli formation (Figure 3) [50]. Sequential deletion of repeat segments suggested that CsgA could make amyloids in the absence of R1-R4 (ΔR1-ΔR4) with comparable lag time, however deletion of R5 (ΔR5) increased the lag time substantially indicating the significance of R5 region in early oligomerization process during CsgA fiber assembly [58]. The specific arrangement of aspartic acid and glycine residues in the repeat regions of R2, R3 and R4 prevent spontaneous aggregation of Csg proteins inside the cell [59]. Owing the capacity for these aspartic acid residues to curtail CsgA amyloid formation, they are termed “gatekeeper residues” (Figure 3) [59].To gain insight into the functionality of individual repeat regions in curli assembly, peptides corresponding to each region were synthesized [54]. The synthetic peptides (R1 to R5) display different degrees of amyloid formation. The peptides from the R1, R3 and R5 regions were found to be highly amyloidogenic and polymerize with faster rates than peptides corresponding to R2 and R4 [54]. CsgA amyloids influence biological functions in the host through specific interactions between the R1/R5 repeat regions and human proteins such as fibronectin and tissue plasmogen activator [60,61].

Inside the bacterial cell, CsgA is synthesized and secreted as an IDP [57]. The amyloid assembly of CsgA on the outer membrane is dependent on minor curli subunit CsgB which serves as a template or nucleator for the initiation of CsgA amyloidogenesis [43]. The detailed genetic, biochemical and microscopic analysis revealed that N and C- terminus of CsgA is required for CsgB templated assembly of CsgA [62].

### 4.2 The minor curli subunit and nucleator protein, CsgB:

CsgB, the minor curli subunit has 30% identity with CsgA at the amino acid level[63]. Under *in vivo* conditions, full-length CsgB is membrane associated and self-polymerize to present a template for CsgA polymerization [43]. Cells with a *csgB* deletion secrete CsgA, although CsgA remains unpolymerized on the cell surface[63]. CsgB is also predicted to be an IDP [49]. Purified CsgB seeds CsgA polymerization under *in vitro* conditions [43,64]. A truncated version of CsgB ($CsgB_{trunc}$) containing four repeats units (R1-R4) was used to understand the aggregation mechanism of CsgB under *in vitro* conditions. *In vitro,* $CsgB_{trunc}$ makes amyloids similar to CsgB, although it does not complement a *csgB* mutant [43] in cells because $CsgB_{trunc}$ does not associate with the outer membrane and is not positioned correctly to facilitate CsgA nucleation [43].

Like CsgA, CsgB also contains five imperfect repeats with a slight variation in the position of consensus sequence as compared to CsgA[43]. Sequence alignments reveal high degree of conservation in first four repeats (R1-R5) however, the fifth repeat (R5) is less conserved as compared to CsgA [43]. Biochemical and biophysical studies on different fragments of CsgB indicate that the repeat regions have redundancy and the protein still self-assemble *in*

*vitro* after deletion of individual repeats [62]. An attractive model of CsgB-mediated nucleation of CsgA, suggests that CsgB rapidly forms an amyloid-like template on the cell surface that can template the folding of CsgA [64].

Curli amyloid formation can be specified to the cell surface because that is the place where the CsgA protein can interact with the CsgB nucleator. However, both CsgA and CsgB are IDPs and have some propensity to aggregate within the cell [51,65]. It is not surprising that a sophisticated network of chaperones and protein-degradation machinery is in place to keep Csg proteins from premature aggregation within the cell [34,43,66]. In the following sections, we will discuss various chaperone systems that keep the disordered Csg proteins on the right track.

## 5. Chaperones in *E. coli:* keeping the disordered Csg proteins at right place and time

### 5.1 Traditional molecular chaperones; Dna K, Hsp33 and Spy proteins:

Chaperones and chaperone-like proteins in cytoplasm and periplasm can prevent the inappropriate and premature aggregation of curli proteins. General chaperones such as DnaK, Spy and Hsp33 can prevent CsgA amyloid formation *in vitro* [66]. DnaK can transiently inhibit the aggregation of CsgA, even in the absence of DnaJ and ATP hydrolysis [66]. Similarly, the Hsp33 “holdase” can prevent CsgA amyloid formation [44]. Hsp33 is activated by oxidative stress and the oxidized form of Hsp33 is a more potent amyloid inhibitor than the reduced form [67]. The periplasmic holdase chaperone, Spy, stabilizes the monomeric soluble form of CsgA[66,68]. However, the role that these general chaperones play in keeping Csg proteins from aggregating *in vivo* remains largely unexplored.

### 5.2 A potent anti-amyloid periplasmic chaperone-like protein, CsgC:

CsgC is a periplasmic protein whose role in curli assembly is to inhibit CsgA and CsgB aggregation inside the cell [46].By preventing CsgA and CsgB from aggregating inside the cell, CsgC mitigates potential cellular toxicity [46]. CsgC is a β-sheet rich protein with a high degree of stability[69]. Strikingly, CsgC inhibits CsgA amyloid assembly at sub-stoichiometric ratios. Interestingly, CsgC can prevent amyloid formation by other bacterial and human proteins, including α-synuclein[46]. While CsgA and CsgB has 30% sequence identity, CsgA and α-synuclein share little significant sequence identity [46]. The only partially shared stretch of amino acids appears in each repeating unit of CsgA(R1-R5), and only one time in α-synuclein [46] Deletions or mutations in the shared stretch from α-synuclein renders CsgC non-responsive to α-synuclein aggregation [46]. CsgC exhibits client specificity as it is not able to prevent Aβ aggregation [46]. The mechanistic insights into the CsgC inhibitory activity highlights the role of electrostatic interactions and presence of specific amino acids for the potency of CsgC as an amyloid inhibitor [70]. Recently, a super-resolution imaging technique found that CsgC could ‘cap’ the growing CsgA fiber tip [71]. The detailed mechanism of inhibition and specificity of CsgC still remains to be understood. However, the discovery has opened avenues to explore the presence of CsgC like proteins in other kingdoms that can serve as amyloid inhibitors [61,70,72]. In sum, CsgC helps maintain a dynamic population of soluble and disordered CsgA and CsgB in the

periplasm and is an elegant example of how bacteria have evolved a well-developed machinery to mitigate premature amyloid formation [73].

## 6. Accessory proteins: Disordered to ordered transition from inside to outside of the cell

In *E. coli,* controlled translocation of amyloidogenic curli subunits to the cell surface is achieved *via* several of the Csg (**c**urli **s**pecific **g**ene) proteins.

### 6.1 Membrane tethered, CsgF:

CsgF is a surface exposed, membrane-associated oligomeric protein [74,75]. A recent NMR study found that CsgF lacks a stable tertiary structure and contains exposed hydrophobic surfaces [76]. However, CsgF attains a stable conformation after interaction with CsgG and CsgB [76]. Cells deficient in *csgF* are less capable of polymerizing CsgA into amyloid [75]. Therefore, CsgF is itself an IDP that must function in concert with other Csg proteins to ensure the ordered assembly of intrinsically disordered CsgA and CsgB into curli amyloids [75]. The primary sequence of CsgF contains a high percentage of glutamine and asparagine residues, similar to CsgA and CsgB, which may act as CsgG specific export signal [51]. The C-terminus of CsgB interacts with dimeric CsgF and this interaction apparently promotes CsgB to adopt a nucleation competent conformation [76]. It is also possible that CsgF works directly on CsgA to promote amyloid formation, although this has not been directly demonstrated in cells.

### 6.2 The secretion pore, CsgG and CsgE:

The outer-membrane secretion channel is composed of a nonamer of the CsgG protein, which is ‘capped’ on the periplasmic side by a multimer of CsgE [51,77]. The nine subunits of CsgG form an oligomeric transport complex that gives rise to a 36-stranded β-barrel which traverses the bilayer and is connected to a cage-like vestibule in the periplasm [77]. The channel formed by the CsgG complex is 2nm wide, and is suited to export unfolded proteins [77]. The three known substrates of the CsgG secretion complex are CsgA, CsgB and CsgF, each of which is putatively disordered during secretion [51,77]. The CsgG mediated secretion of CsgA is dependent on the N-terminal 22 amino acids of soluble CsgA [51]. These residues are required for secretion and assembly of curli fibers but do not become the integral part of the fibers [51]. Structural and functional analyses of CsgG imply that it is a general secretion channel that transports protein on a diffusion based, entropy-driven transport mechanism [77]. The overexpression of CsgG results in outer-membrane permeability to antibiotics such as erythromycin [51].

CsgE acts as efficiency factor for CsgG by capping the periplasmic side of the vestibule and allowing translocation of Csg proteins to the cell surface [65]. CsgE also prevents non-specific translocation of small proteins and large molecules through CsgG pore, thus acting as a regulatory gateway to the otherwise permissible CsgG pore [65,74,78]. CsgE also inhibits CsgA polymerization *in vitro* suggesting that it may help keeping CsgA in soluble form in the periplasm [65,79]. Hydrogen-deuterium amide exchange revealed that CsgE exists in a dynamic ensemble of monomer and oligomers [80]. A recent NMR structure of a

mutant of CsgE (W48A/F79A) highlights the detailed structural properties and localization with CsgG [74]. The NMR structure suggest that the N and C terminal of CsgE are flexible and may help in the interactions with CsgG [78]. According to the 'head-center' model, CsgE-CsgG interactions are driven by electrostatic forces where the flexible and negatively charged C-terminal of CsgE interacts with periplasmic domain of CsgG which is rich in positive residues [74]. The positively charged N-terminal of CsgE electrostatically interacts with soluble disordered CsgA, either as monomer or oligomeric form [74,78]. Thus, CsgE serves as a chaperone-like protein and a cap that allows an effective unidirectional transport of CsgA from periplasm to outer membrane [78].

### 6.3 The master regulator; CsgD:

CsgD is a key curli transcriptional regulator protein that belongs to FixJ/LuxR/UhpA transcription factor family [81]. It is the key modulator that regulates the csg*BAC* and csg*DEFG* operons required for the synthesis, secretion and assembly of curli components [82]. Numerous transcription factors and other parameters like amount of oxygen, temperature, osmolarity, starvation, high cell density plays critical role in regulating CsgD expression and thus indirectly influence *csgBAC* expression [83]. Transcription of CsgD is regulated by cAMP-CRP (cyclic AMP-catabolite repressor protein) complex and small RNAs [84,85]. The exact stimulus required for CsgD activation is not yet known but it is believed that the activated CsgD is phosphorylated at N-terminus by an unknown mechanism. The activation of CsgD governs the expression of curli and accessory proteins [84].

## 7. Curli and biofilm formation

Curli along with cellulose and extracellular DNA makes an integral part of extracellular matrix of biofilm in *E. coli* [34]. The extracellular matrix is a highly organized three-dimensional structure that protects bacteria from environmental stress and aids in adherence to biotic and abiotic surfaces [48,86]. Presence of biofilm renders bacteria resistant to antibiotics and host defense [87]. Furthermore, biofilm formation within the host is implicated in serious and persistent infectious diseases, including cystic fibrosis, chronic otitis media and urinary tract infection (UTI) [87]. Curli are also capable of evading host immune system and cause inflammation [88-90]. The specific and complicated interactions of curli with host environment and immune system explains the vital role of functional amyloids in host-bacterial interactions [88].

Although beneficial to bacteria, biofilms pose a great threat to humans since it is almost impossible to completely eradicate mature biofilms from biotic and abiotic surfaces. Preventing curli assembly is one of the most attractive strategies to prevent biofilm formation and subsequent damage [79]. Since curli shares similar biochemical and biophysical properties with disease-related amyloids, it is also a well suited model to design and develop general amyloid inhibitors [79,91,92].

## 8. Conclusion

The discovery of functional amyloids has greatly expanded our view of amyloid biology. Clearly, amyloid formation is not always deleterious from the cell's perspective. However, there is still much to learn about amyloid formation and toxicity associated with amyloids. Many IDPs undergo liquid/solid phase transition to form hydrogels which are "solid-like amyloids" to prevent toxic effects of amyloids and imparts functional role. The most well studied functional amyloid, curli in *E. coli* has provided insights into how cells make amyloids efficiently at the right time and place, thus eliminates the changes of intracellular aggregation and cytotoxicity. Studies on curli not only enable us to understand the process of amyloidogenesis, but also provide a fascinating tool to discover new ways to treat amyloid-related diseases.

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## Biography

Neha Jain studied microbiology at Maharani's college and received Bachelor's and Master's degree from the University of Rajasthan, Jaipur. Later she pursued PhD in Biological Sciences from the Indian Institute of Science Education and Research (IISER Mohali) with Prof. S. Mukhopadhyay. After finishing her PhD in 2013, she joined Prof. M. R. Chapman's lab at the University of Michigan (Ann Arbor campus) for postdoctoral training. In 2018, she returned back to India. After a short stint as Assistant Professor at Ahmedabad University, she has joined the faculty of Bioscience and Bioengineering at the Indian Institute of Technology Jodhpur. Her lab is interested in understanding the interactions between human and bacterial amyloids and their role in pathophysiology of neurodegenerative diseases.

Matthew R. Chapman received his Bachelor's degree in microbiology from the University of Nebraska at Omaha. He then moved to the Indiana University to pursue PhD with Prof. C. Kao. He joined Prof. S. J. Hultgren's lab for postdoctoral studies where he discovered that the major proteinaceous component of the biofilm extracellular matrix belonged to a class of structurally conserved ordered aggregates called amyloid. In 2003, he started his lab at the

University of Michigan, Ann Arbor. His lab has continued to work on the biogenesis and regulation of functional amyloids in bacteria.

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## Highlights

- IDPs play pivotal role in biological functions.
- IDPs are prone to collapse and form structured aggregates called amyloids.
- Curli is a bacterial functional amyloid.
- Curli is a major constituent of extracellular matrix in bacterial biofilms.
- Curli biogenesis is spatio-temporally regulated to mitigate intracellular toxicity.

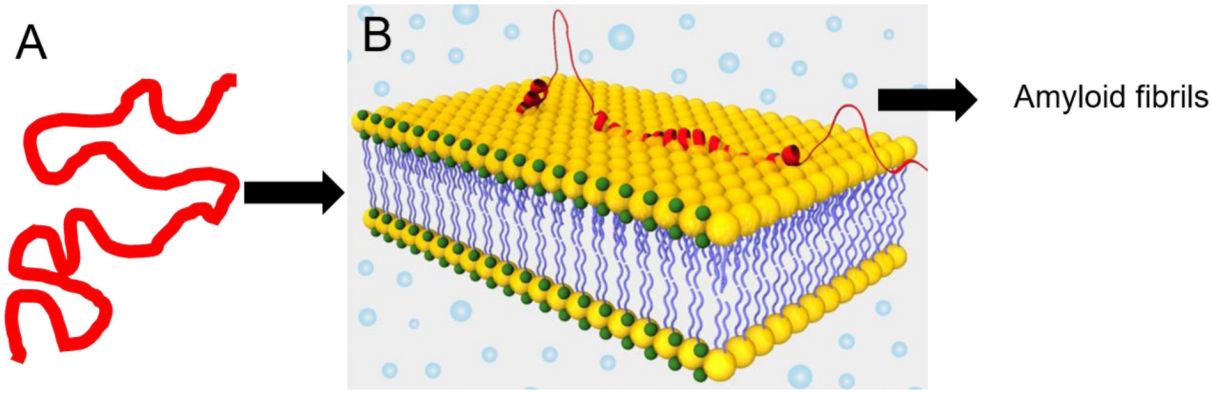


**Figure 1:**
Conformational switch of α-synuclein (AS; an archetypal IDP) induced by lipid bilayer. A. Native unfolded structure of AS in solution. B. Predominantly helical structure in the presence of lipid bilayer which is prone to aggregate and form amyloid fibrils.

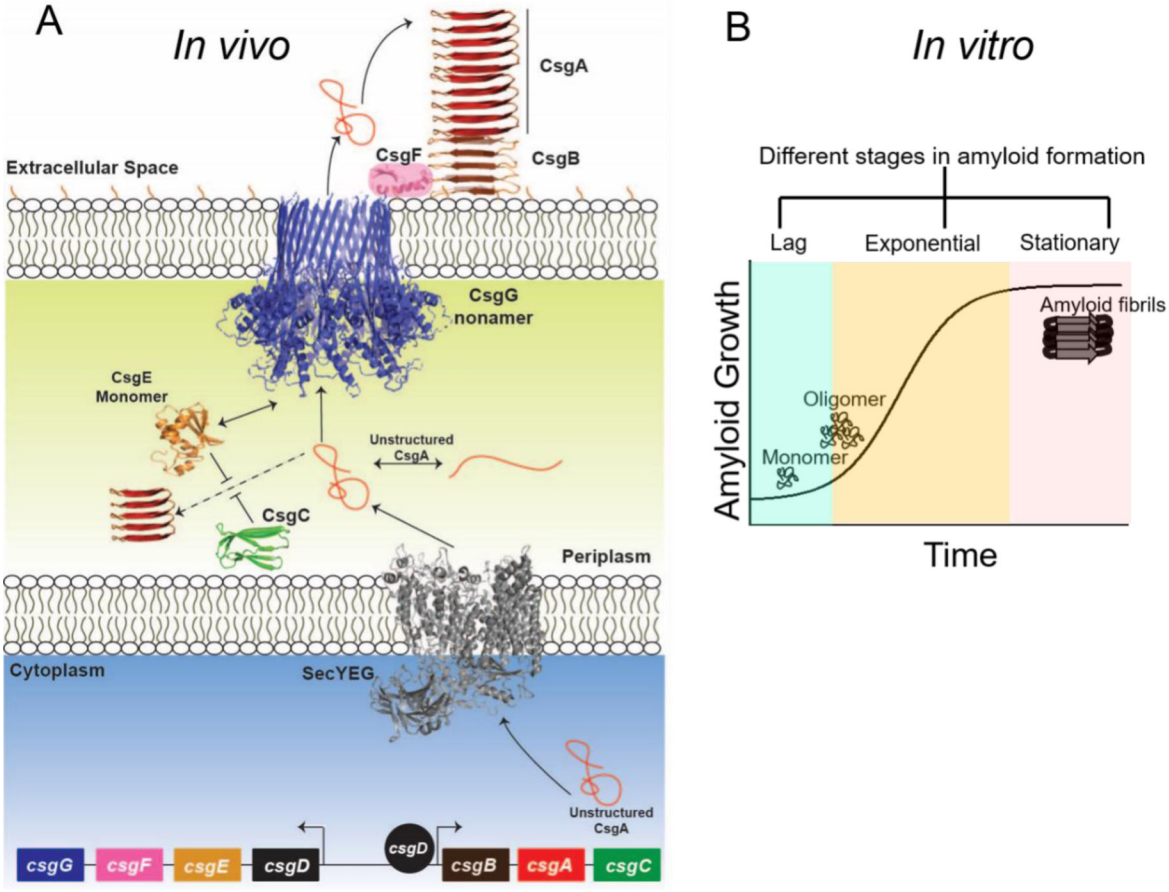


**Figure 2:**

The biogenesis model of curli *in vivo* and *in vitro.* A. Controlled translocation of disordered monomeric CsgA and CsgB on cell surface. CsgA self assembles into fibrils on nucleator CsgB amyloids. B. *In vitro* amyloid formation of CsgA follows a nucleation-dependent aggregation pattern.


**Figure 3:**

The details of repeat regions in CsgA amino acid sequence. The full length CsgA comprises of N-22 peptide sequence and five repeat units (R1-R5). The Gln residues at position 49 and 139 and Asn residues at position 54, 144 (all highlighted in cyan) are essential for CsgA amyloid assembly. The “gatekeeper residues” in regions R2, R3 and R4 (encircled in violet) prevent spontaneous aggregation. The conserved amino acids across repeat units are boxed together.