Protein aggregation in bacteria: the thin boundary between functionality and toxicity

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Misfolding and aggregation of proteins have a negative impact on all living organisms. In recent years, aggregation has been studied in detail due to its involvement in neurodegenerative diseases, including Alzheimer’s, Parkinson’s and Huntington’s diseases, and type II diabetes – all associated with accumulation of amyloid fibrils. This research highlighted the central importance of protein homeostasis, or proteostasis for short, defined as the cellular state in which the proteome is both stable and functional. It implicates an equilibrium between synthesis, folding, trafficking, aggregation, disaggregation and degradation. In accordance with the eukaryotic systems, it has been documented that protein aggregation also reduces fitness of bacterial cells, but although our understanding of the cellular protein quality control systems is perhaps most detailed in bacteria, the use of bacterial proteostasis as a drug target remains little explored. Here we describe protein aggregation as a normal physiological process and its role in bacterial virulence and we shed light on how bacteria defend themselves against the toxic threat of aggregates. We review the impact of aggregates on bacterial viability and look at the ways that bacteria use to maintain a balance between aggregation and functionality. The proteostasis in bacteria can be interrupted via overexpression of proteins, certain antibiotics such as aminoglycosides, as well as antimicrobial peptides – all leading to loss of cell viability. Therefore intracellular protein aggregation and disruption of proteostatic balance in bacteria open up another strategy that should be explored towards the discovery of new antimicrobials.

Protein misfolding and aggregation

Protein aggregation arises as a result of protein misfolding in response to mutation, post-translational modification, or stressful environmental change. Macromolecular crowding due to concentrations of macromolecules inside the cell as high as 340 g l−1 (Cheng et al., 2007) exacerbates the problem. In addition, high protein expression, for example caused by impaired protein degradation, can also facilitate aggregation of non-native polypeptide chains (Markossian & Kurganov, 2004). Protein folding is an inefficient stochastic process that often fails and the resulting misfolded proteins can inappropriately expose hydrophobic surfaces that are normally buried in the interior of the protein, leading to non-native conformations that are able to interact with each other to form aggregates (Fig. 1). Protein aggregates are often implicitly classified as highly structured amyloids or amorphous aggregates. However, it became apparent that such classification is oversimplified and many proteins can form a spectrum of aggregate morphologies depending on the prevailing physico-chemical conditions. Regardless, most types of aggregates tend to be insoluble and metabolically stable under physiological conditions; moreover intramolecular β-sheets have now been recognized as a common structural element of aggregates that are shared by amyloid fibrils and many amorphous aggregates (Tyedmers et al., 2010). Consequently, this type of aggregation is commonly called amyloid-like or β-aggregation. Although several other aggregation mechanisms exist, the field of protein aggregation research has accepted the view that the formation of β-structures by short aggregation-prone polypeptide stretches, called APRs, is the most commonly occurring and most biologically relevant protein aggregation mechanism. As a consequence, native protein folding of globular proteins protects from aggregation, as the APRs are hidden...
within the core of the folded protein. However, during the lifetime of a protein these APRs may become exposed to a trigger aggregation. β-Aggregation involves nucleation via the formation of intermolecular β-sheets that gives rise to the core of an aggregate (Nelson et al., 2005; Makin et al., 2005). It is thought that intermolecular β-structures derive their great thermodynamic stability from the high degree of hydrogen bond formation of the backbone of the polypeptide chain, but the amino acid side chain configuration will strongly influence the rate of fibril formation (DuBay et al., 2004). In practical terms, under a given set of condition it is the side chains, i.e. the specific amino acid sequence, that will determine if aggregation will occur on biologically relevant timescales. Amyloid-like aggregation is driven by a balance between protein intrinsic parameters such as hydrophobicity, electrostatics and β-sheet propensity and extrinsic factors (Chiti & Dobson, 2009) such as temperature, pH, ionic strength and protein concentration (Sarell et al., 2013). Despite their heterogeneity and source, most polypeptide aggregates display similar features, including poor solubility in aqueous or detergent solvents, aberrant subcellular or extracellular localization, non-native secondary structure (Fink, 1998), binding to certain sensor dye molecules such as Congo red and thioflavin-T and as mentioned above cross-β-sheet structure (Garcia-Fruitós et al., 2005). The degree of β-sheet organization is, however, variable in the different aggregate forms, and the highest degree of order is commonly thought to be associated with amyloid fibrils, in which the β-sheets run perpendicular to the fibril axis (Tyedmers et al., 2010), which can extend to several micrometres in length. The amyloid-fibril structure is employed throughout the natural world as a nanomaterial for its great tensile strength and adhesiveness, such as in spider silk, but strikingly, it is also specifically the precursors of amyloid fibrils that are thought to mediate cytotoxicity, in particular to neurons, in aggregation-associated diseases such as Alzheimer’s (Benilova et al., 2012). In contrast to amyloids, amorphous aggregates do not have regular microscopic structure and they are generally non-toxic forms. The aggregation of proteins into amorphous aggregates results in a loss of function (Reumers et al., 2009; Rousseau et al., 2006a) and consequently has not been observed as a way to generate functional materials. Many years of intense in vitro study of peptide and protein aggregation have shown that almost all proteins can form amorphous aggregates when induced at high concentration, but under a set of native-like conditions only some are able to form highly ordered β-rich amyloid fibrils (Maurer-Stroh et al., 2010).

In terms of reaction kinetics, aggregate formation usually goes through a slow nucleation step during which a ‘seed’ is formed consisting of a relatively small number of molecules, from which larger aggregates can grow through monomer addition or seed fusion. This process of subsequent addition of units to the growing polymer is usually very fast and energetically favourable. The protein aggregation reaction is essentially homotypic and aggregates are usually strongly enriched in a single protein, whether they are bacterial inclusion bodies (IBs) or disease-associated protein deposits in patient tissues. However, aggregation of one protein in a cell can increase the aggregation of unrelated proteins, for example by depleting essential cellular factors required to support proper protein folding (Bence et al., 2001). Moreover, heterologous induction of protein aggregation, known as ‘cross-seeding’, has also been described in vitro, e.g. between mammalian huntingtin and rhodopsin (Steffan et al., 2000), or between the amyloid β protein (Aβ) and α-synuclein (Ono et al., 2012). Although it remains unclear how the cross-seeding actually works on a structural level, these data highlight that it is critical for the cell to eliminate or at least strictly compartmentalize aggregation of any protein in order to prevent a wide-spread collapse of protein solubility.

**Protein homeostasis and bacterial cellular responses to aggregation**

Protein aggregation constitutes a major challenge to the cell: it can serve physiological functions, but unchecked it can also lead to rapid cell death due to the loss of function of a myriad of proteins with essential functions (Olzscha et al., 2011) and even if the bacteria succeed in detoxifying the aggregates by formation of IBs, it implies a major fitness cost. During their evolution bacteria developed different strategies to avoid the detrimental effect of accumulating misfolded proteins. The volume of the cytosol of *Escherichia coli* MC4100 (0.67 μm³) is ten-fold higher than the volume of its periplasm (0.065 μm³) and it was shown that the periplasmic proteins seem to be protected from aggregation due to specific sequence characteristics (de Groot & Ventura, 2010). In the cytosol, macromolecular crowding can have large effects on the interaction of all types of molecules: it can lead to initiation of spontaneous non-specific aggregation of proteins that are less represented in the cell or depletion of functionally important proteins. The cytosol therefore contains a highly sophisticated protein quality control system to avoid detrimental effects of protein aggregation. This led to the concept of proteostasis, short for protein homeostasis, defined as the cellular state in which the proteome is both stable and functional (Balch et al., 2008), implicating an equilibrium between synthesis, folding, trafficking, aggregation, disaggregation and degradation. Molecular chaperones, through regulated binding and release, can directly assist in correct folding via ATP-dependent mechanisms and protect misfolded proteins from aggregation, while proteases cleave the misfolded forms. Proteases and chaperones together form a protein quality control system of the cell. This proteostasis network is a complex system interlinked with numerous biological pathways, controlled by stress-responsive signalling pathways. For example, molecular chaperones can function under normal conditions and under stress conditions like heat. The name ‘heat-shock protein’ stems from their original discovery as...
heat-responsive elements, and ‘molecular chaperones’ is perhaps better suited as a general name (Hartl et al., 2011). Under normal conditions the expression of heat-shock genes is restricted by chaperones or the proteases themselves, which inactivate the expression activators, or via repressors that require chaperones for their activity. Under stress conditions chaperones and proteases are recruited to misfolded proteins, leading to the expression of heat-shock genes by the transcriptional activators and the inactivation of repressors. This regulatory circuit creates a negative feedback when proteostasis is affected (Guisbert et al., 2004; Mogk et al., 2002). Molecular chaperones prevent aggregation of misfolded proteins by binding to exposed hydrophobic surfaces and in particular to APRs (Rousseau et al., 2006a, b), thereby stabilizing them. If refolding fails, chaperones can usher terminally misfolded proteins towards proteolytic degradation, which constitutes the ultimate clearance mechanism.

Molecular chaperones and proteases can be classified in different families depending on their molecular mass: Hsp70 (in *E. coli*: DnaK), Hsp40 (in *E. coli*: DnaJ), Hsp90 (in *E. coli*: HptG) and small Hsps (in *E. coli*: LpbA, LpbB). Other proteases are classified in the AAA+ family (ATPases associated with diverse cellular activities), including Lon, HsiUV and the group of Clp-caseinolytic protease family (Muchowski & Wacker, 2005). In addition to these, ribosome-associated chaperones exist, such as trigger factor, which binds nascent polypeptides as they come off the ribosomes. Degradation and refolding of insoluble aggregates in *E. coli* proceed in a number of sequential steps, accompanied by DnaK chaperone and by

**Fig. 1.** Schematic representation of some states of protein after synthesis on ribosome. A protein during and after its synthesis folds through different intermediates to its native, three-dimensional structure. In a living cell, the fate of given protein molecule is regulated by molecular chaperones and other quality control mechanisms. Proteotoxic stresses may interfere with folding and increase prevalence of misfolded intermediate species. Overload or lack of quality control network leads to accumulation of toxic aggregated proteins. Aggregates can have different structures, ranging from mostly unstructured, disordered to rich in β-sheet, pre-fibrillar species or well-structured amyloid fibrils. Arrows show that most of the aggregation states are reversible (may be degraded).
the DnaJ and GrpE cofactors and in cooperation with other chaperones such as ClpB, as well as the GroEL chaperone and the GroES co-chaperone (Markossian & Kurganov, 2004). In eukaryotes, the main energy-dependent protease is the 26S proteasome, while in bacteria, the members of the Clp protease family are prominently involved (such as ClpAP, ClpCP, ClpEP, ClpXP, HsUVT) as well as Lon protease and the membrane-bound FtsH protease (Kress et al., 2009). Exposed hydrophobic regions are recognized by Clp-chaperone-proteases, along with Lon protease. It was previously shown that resolubilization of aggregates can be mediated by the ClpB and DnaK system (Schlieker et al., 2002), whilst disintegration of larger aggregates of IBs was shown to be directed by Lon and ClpP proteases in cooperation with the AAA+ unfoldases (Martinez-Alonso et al., 2009). The DnaK–ClpB disaggregation system relies on binding of DnaK to aggregates and disentangling the polypeptides from the aggregate. Then the polypeptides are transferred to ClpB, which further disaggregates it into unfolded polypeptides that can refold spontaneously or with the help of additional chaperones (Liberek et al., 2008). The physiological disassembly of IBs is directed by Lon ATP-dependent protease and ClpP protease. The disaggregation process is started by Lon, which recognizes specific hydrophobic and aromatic stretches in damaged proteins, followed by ClpP binding of the released, reversibly misfolded proteins (Vera et al., 2005).

In addition to active protein quality control by chaperones and proteases, it appears that there exists a relationship between protein concentration and solubility at the genetic level. Proteins with a low tendency to aggregate have high expression levels and vice versa (Castillo et al., 2011; Tartaglia & Vendruscolo, 2009; Jahn & Radford, 2005). Moreover, genes encoding aggregation-prone proteins are under tighter transcription, translation and degradation control than non-aggregation-prone proteins (Gsponer & Babu, 2012). Moreover, it was revealed that in order to minimize the risk of aggregation by exposure of hydrophobic residues, the flanks of APRs are enriched with gatekeeper residues, which slow down the aggregation process. These gatekeepers include charged residues like arginine (R), lysine (L), aspartate (D), glutamate (E) and proline (P) (Rousseau et al., 2006b; Beerten et al., 2012). In spite of these preventive strategies, bacteria are exposed to stress conditions that can lead to accumulation of misfolded or mistranslated proteins. In that case, the cellular protein quality control adapts to the protein damage, depending on its severity, in three distinct but interconnected ways: refolding and degradation mentioned above, or delivery to the designated compartments discussed below (Fig. 2).

**Sequestration into specialized quality control compartments — IBs.** Preserving protein homeostasis involves numerous parallel strategies that aim at either refolding, degrading or sequestering misfolded polypeptides. The latter implies spatial compartmentalization of cellular quality control and misfolded proteins into inclusions, which helps the cell to cope with the overload of misfolded proteins and regulate their inheritance. In bacteria the aggregated and misfolded proteins were shown to move to the poles of the cell, forming IBs that function as a temporary zone for cellular detoxification (Dougan et al., 2002; Steffan et al., 2000). The sequestration points towards evolutionary pressure against the inheritance of aggregates to progeny because IBs distribute asymmetrically during cell division and accumulate in the daughter cell harbouring the older poles (Lindner et al., 2008). The specific pole localization allows rapid elimination of the aggregated proteins from most of the cell population. Presence of IBs is directly associated with ageing and a decrease of cell productivity (Ventura & Villaverde, 2006). Therefore, IB formation might be a sophisticated mechanism to reduce the potentially toxic, partially folded monomers or small oligomers, of which the solvent-exposed APRs may interact with cellular components leading to exhaustion of the proteostatic machinery. The chaperones GroEL and DnaK are major antagonists of IB formation (Carrió & Villaverde, 2003); DnaK prevents inclusion body development by reducing the aggregation degree of misfolded protein, whereas GroEL drives the protein transit between the soluble and insoluble cell fractions and participates in IB formation (Markossian & Kurganov, 2004).

**IBs — large aggregates.** At low magnification, all IBs share a common structure; they are very condensed, insoluble particles that can be found in both the cytoplasmic and periplasmic space of bacteria. Originally, IBs were considered as non-specific protein aggregates; however, deeper insight into their morphology showed that bacterial IBs display amyloid-like properties (Carrió et al., 2005). The structural data suggest that newly formed β-sheet architecture in IBs is stabilized by a network of hydrogen bonds, resulting in tightly packed, extended intermolecular β-sheets (Ventura & Villaverde, 2006). IBs satisfy two requirements out of three to be considered as amyloids: they bind to thioflavin-T and Congo red dyes, and they have a high β-sheet content. However under atomic force microscopy, IBs appear amorphous. This observation does not dismiss the fact that IBs might indeed have an amyloid structure, because even amyloids do not incorporate their entire polypeptide length into the highly packed β-sheet structure as seen in yeast prions (Morgan et al., 2008). The secondary structure content analysis of IBs confirmed that there are fibrillar and non-fibrillar regions included (Balguerie et al., 2003). Moreover comparison of IB formation with an amyloid β-formation revealed a common formation mechanism: primary establishment of an inter-backbone, hydrogen-bonded network that stabilizes β-sheet-rich fibrillar structures and drives the aggregation force. The ability of IBs to seed amyloid formation is the most compelling evidence to support the amyloid nature of IBs (de Groot et al., 2009). There is a high specificity that accompanies this reaction; IB recognizes and incorporates only homologous polypeptides (Carrió et al., 2000) similar to what is observed with amyloid-fibril seeds.
Toxicity of aggregates

Common mechanisms of amyloid pathogenesis. Several lines of evidence suggest unrelated protein aggregates may share common mechanisms of toxicity. Most publications relate to the pathogenesis of amyloids in degenerative diseases, but the similarities between eukaryotic and bacterial amyloids and experimental data in bacteria make it possible to speculate on general aggregate toxicity mechanisms in bacteria. There is evidence that the higher the amount of cross-β-structure within an aggregate the less toxic it is, indicating that loosely packed conformations are the most toxic ones (de Groot et al., 2009). Furthermore, since protein function is determined by the three-dimensional structure, the fact that amyloids share generic structures implies that they may also share a common pathological function (Glabe, 2006). This hypothesis is strongly supported by the finding that soluble oligomeric aggregates of differing peptides are equally recognized by polyclonal antibodies whereas the same antibodies are unable to recognize the fibrillar aggregates of same protein (Kayed et al., 2003). These common properties of eukaryotic amyloid oligomers and the pathway of amyloid-fibril formation may explain similarities in cytotoxic behaviour of mammalian non-related amyloids in different diseases.

A large amount of data on aggregation in mammalian cells led to the formulation of the ‘channel hypothesis’, which is based on the observation that amyloidogenic proteins, such
as Aβ, α-synuclein, polyglutamine and IAPP, are able to form pores or single channels in membranes in vitro (Kagan et al., 2001; Quist et al., 2005; Lashuel & Lansbury, 2006). The small annular Aβ and α-synuclein closely resemble the cytolytic β-barrel pore-forming bacterial toxins such as z-toxin, latratoxin and aerolysin, suggesting a common mechanism of membrane interactions (Hotze et al., 2002). Other groups also confirmed the channel hypothesis, as it was for example reported that polyglutamine (involved in Huntington’s disease) could form large, long-lived and non-selective ion channels in planar phospholipid bilayers (Hirakura et al., 2000). Theoretical calculations showed that polyglutamine (PG) is able to form a stable structure containing a cylindrical pore of 3.7 Å diameter (Monoi, 1995). Later these in silico results were empirically confirmed by showing that PG indeed forms relatively small, cation-selective, proton-permeable channels (Monoi, 1995). On the contrary, another group (Green et al., 2004), using a dye release assay, confirmed that the amyloidogenic human amylin peptide causes membrane disruption, but atomic force microscopy revealed that this did not occur by the formation of defined pores. Despite this discrepancy, there are consistent results showing that amyloids permeabilize cell membranes causing leakage of sequestrated Ca2+ and disrupting intracellular signalling (Demuro et al., 2005). The intracellular calcium concentration is tightly regulated in both prokaryotes and eukaryotes, ranging from 100 to 300 nM (Dominguez, 2004). Calcium is involved in many other signalling pathways either directly or indirectly, including pathways that lead to apoptosis. In bacterial cells, calcium ions are involved in the maintenance of cell structure, motility, transport and cell differentiation processes such as sporulation, heterocyst formation and fruiting body development (Herbaud et al., 1998). There are a wide range of data published on cellular responses to the intracellular redox status and free Ca2+ levels in cells exposed to toxic aggregates (Milhavet & Lehmann, 2002). Changes have been observed consisting of a sharp increase in the quantity of reactive oxygen species, reactive nitrogen species, lipid peroxidation, deregulation of NO metabolism, protein nitrosylation and upregulation of haem oxygenase-1, a specific marker of oxidative stress (Choi et al., 2000; Guentchev et al., 2000; Hyun et al., 2002). These data suggest that aggregates can cause overall cellular dysfunction through an increase in macromolecular crowding and induce oxidative and nitritative stresses. These stresses in consequence may lead to membrane lipid peroxidation resulting in membrane permeability impairment and possibly a failure in regulating plasma membrane proteins, such as receptors and ion pumps.

**The effect of aggregates on bacterial membranes.** A recent study provides the first experimental evidence that aggregation of recombinant cytoplasmic proteins affects directly the lipid moiety of bacterial membranes (Ami et al., 2009). It was shown that protein aggregation and misfolding induce changes, such as carbonylation or oxidation of host-specific proteins, a reduction in membrane permeability and rearrangements of its lipid components. The authors also point out that misfolded proteins and soluble aggregates and not the large insoluble aggregates are responsible for these modifications. This is consistent with previously mentioned evidence that the most toxic forms of amyloids are pre-fibrillar assemblies, so-called toxic oligomers. It was shown that pre-fibrillar aggregates of proteins that are unrelated to amyloid-related diseases can also be toxic (Bucciantini et al., 2002) together with activation of IbPAB chaperones (known as protein aggregation markers); recombinant protein aggregates stimulate the levels of SOD (superoxide dismutase) and E3 (dihydrolipolyl dehydrogenase) – proteins involved in antioxidant metabolism and prevention of lipid peroxidation (Amijee et al., 2009; Su et al., 1999). Moreover the accumulation of OmpA (outer-membrane protein A, responsible for membrane stabilization) was also found in cells containing recombinant glutathione-S-transferase aggregates (Amijee et al., 2009; Villa et al., 2009). There was also a noticeable increase in the levels of other membrane proteins such as LamB, OmpF and TolC in *E. coli* (Villa et al., 2009). It was suggested that bacterial membranes are the key factors in sensing protein aggregation and they undergo lipid rearrangements depending on the aggregation state of the accumulating protein. Furthermore these rearrangements might have a modulating effect on the trans-membrane traffic of small solutes, like nutrients or metabolites. Taken together, these results fit well with the channel hypothesis and could be summarized (Fig. 3) in the following scenario of aggregate-induced toxicity:

- Environmental factors, (stress, antibiotic treatment, protein overexpression) may lead to exposure of hydrophobic stretches of globular proteins which can seed aggregation and lead to oligomeric aggregate or pre-fibrillar amyloid development
- Oxidative stress is induced, including production of free radicals resulting in damage to other cellular proteins
- The proteolytic machinery of the cell is involved: proteases and chaperones overload, molecular crowding effects further promote aggregation
- Lipid peroxidation and membrane lipid rearrangements that result in overexpression of membrane proteins, porins, Toll-like receptors and ion pumps
- Destabilization of ion pumps, Ca2+ leakage and pore formation
- Apoptotic or necrotic cell death

The formation of channels/pores appears to be the end point of this rapid and simultaneous process. This perhaps is the reason why many researchers focused solely on pore formation. Membrane disruption would then be a late consequence of amyloid pathogenesis; nevertheless the importance of the initial perturbations of fundamental cellular responses should not be underestimated.
Functional protein aggregation in bacteria

Despite the fact that they can be toxic, it has become clear that aggregates also have a functional role in bacteria. Bacterial fibres, called curli, are one of several examples of functional amyloids. Curli are components of the extracellular matrix that is required for biofilm formation. The minor curli subunit CsgB provides an amyloid-like template that initiates the polymerization of major curli subunit CsgA. The pre-formed amyloid seed CsgA forms the fibre tip that will grow after interaction with soluble CsgA (Wang et al., 2007). Interestingly the functional CsgA polymerization involves the formation of a transient species similar to that produced by other amyloidogenic proteins such as Aβ, synuclein, islet amyloid polypeptide, insulin, lysozyme, and polyglutamine, involved in human aggregation diseases (Kayed et al., 2003; Wang et al., 2007). Other pathogens such as E. coli and Salmonella spp. generate amyloid that gives them an advantage in colonization and tissue invasion. In E. coli, curli amyloid-fibre formation confers the ability to bind tissue-type plasminogen activator and generate plasmin. Amyloid-mediated plasmin activation has been hypothesized to enhance bacterial cell invasion and virulence via breakdown of the extracellular matrix (Fowler et al., 2007).

It is known that the capacity of Staphylococcus aureus to form biofilms on host tissues and medical devices is one of the major virulence traits underlying persistent infections. Biofilm formation requires adhesion proteins that allow attachment of cells to surfaces following intercellular aggregation that is mediated by intercellular adhesins. Additionally to biofilm forming, the aggregation capacity of fibrinogen-binding surface proteins of Staphylococcus aureus serves as a protection against phagocytosis (Merino et al., 2009).

Some of the bacterial species use functional amyloids to form spores that protect against environmental factors and help in dissemination of bacteria into the environment.
The amyloid properties make the spores an extremely stable structure, highly resistant to pH, heat and radiation extremes. For example, *Bacillus subtilis* sporulation protein TasA (Stöver & Driks, 1999) either can be secreted into the culture medium early in sporulation or can also be incorporated into the spore. It was shown that TasA protein has antibacterial activities providing *B. subtilis* endospore a competitive advantage in colonization (Stöver & Driks, 1999). The antibacterial properties of TasA were speculated to be due to the ability of TasA to form small oligomeric aggregates, recognizable by A11 antibody (marker for pre-fibrillar aggregates) (Romero et al., 2010). The endospore shield is not the only function of TasA protein, which also serves as a major component of *B. subtilis* biofilm matrix. *B. subtilis* makes matrix-encased biofilms on the surface of agar plates as well as robust floating biofilms (pellicles) at the air–liquid interface of standing cultures. The TasA protein forms amyloid fibres and the formation of these fibres is essential for the integrity of the extracellular matrix and thus the biofilm (Romero et al., 2010).

The chaplins (coeliac hydrophobic aerial proteins), decorating the surface of Gram-positive soil bacterium *Streptomyces coelicolor*, are another example of functional amyloids with multifunctional properties (Sawyer et al., 2011). The chaplins are a family of eight secreted proteins (ChpA–H) that promote cellular differentiation in *Streptomyces coelicolor* and help to adapt the surface characteristics during the life cycle of the bacterium. The chaplins are proposed to act as surfactants by lowering the surface tension at the aqueous colony surface of the aerial hyphae, which are vegetative spores of *Streptomyces coelicolor* formed upon starvation. It was shown that ChpH chaplin was the major contributor to aerial hypha development, and structural analysis of the chaplin proved that ChpH indeed possess two amyloid domains, an N- and a C-terminal domain. The C-terminal ClpH domain contains a sequence which is critically important for effective amyloid polymerization and promotion of aerial hyphae (Capstick et al., 2011).

Many strains of the important human pathogen *Streptococcus pyogenes* form cell clusters and this property is crucial for its adherence, resistance to phagocytosis and its virulence. *Streptococcus pyogenes* expresses multiple surface proteins, including the antiphagocytic M-protein and immunoglobulin-binding proteins, such as H-protein, which play an important role in self-association. The key aggregating residues of protein H and homologous protein M1 were identified and shown to reside in the sequence of 19 amino acid residues designated AHP. This sequence was found to facilitate bacterial clustering through coiled-coil AHP interactions of neighbouring bacteria. Moreover the synthetic homologue of AHP was shown to fully inhibit clustering of bacteria and reduced its survival and virulence in mice (Frick et al., 2000). *Streptococcus pyogenes* was shown to maintain the balance of cell clustering depending on the growth conditions: during undisturbed growth the expression of surface proteins H and M1 reaches a maximum which corresponds to an aggregated state (Berge et al., 1997; Chaussee et al., 1997). In contrast, starvation or stress conditions turn on the expression of streptococcal cysteine proteinase (SCP), which efficiently cleaves protein H and M1 at the bacterial surface, dissolves the aggregates and allows dispersion of neighbouring cells to more favourable environments (Berge et al., 1997).

Another pathogen that exploits aggregation as its virulence mechanism is *Bordetella pertussis*, a causative agent of whooping cough. The filamentous haemagglutinin (FHA) is a major adhesin that can be both surface associated and secreted, and FHA–FHA aggregation is a contributory mechanism of virulence in that strain (Menozzi et al., 1994). Similarly, many mycobacterial species use surface-associated haemagglutinin to mediate aggregation and adhere to epithelial cells (Menozzi et al., 1996). This binding can be specifically inhibited by sulfated carbohydrates which could be a good way to provide the basis for the development of new therapeutic and prophylactic strategies against mycobacterial diseases.

Aggregation was shown also to be a mechanism for many bacterial toxins, for example aerolysin, a toxin responsible for the pathogenicity of *Aeromonas hydrophila*. Aerolysin must aggregate prior to disruption of erythrocytes, as it binds to erythrocyte protein glycophorin and oligomerizes before inserting into the membrane (Garland & Buckley, 1988). Incubation of aerolysin with glycophorin purified from human erythrocytes caused aggregation and complete inactivation. Another bacterial toxin, parasporin, produced by *Bacillus thuringiensis* shows homology with aerolysin and similarly aggregates into oligomers on the plasma membrane of mammalian cells without being dependent on the presence of cholesterol (Okumura et al., 2011). Besides using amyloid structures for physiological functions and virulence, bacteria use toxic amyloid intermediates to defend themselves. The examples of such structures are hairpin proteins (produced by *Xanthomonas* and *Pseudomonas* species) (Otzen & Nielsen, 2008) and microcin (produced by *Klebsiella pneumoniae*). Although the exact mechanism of cytotoxicity of these proteins continues to be studied, it has been correlated with toxicity of pre-amyloid oligomeric intermediates (Bieler et al., 2005). The oligomer of microcin protein E492 was shown to kill bacteria by forming ion-permeable pores in their cytoplasmic membrane. Interestingly, aggregation of microcin into amyloid fibrils leads to loss of its antibacterial activity, which also has been confirmed to be the case for TasA protein, described earlier in this paragraph. Therefore the balance between aggregation states of proteins is clearly utilized by bacteria as a way to regulate many physiological functions.

Maintaining the balance between aggregation and function. The co-existence of functional and detrimental forms of aggregation raises the question of how the bacterial cell balances the non-functional and beneficial effects of protein aggregation. It has been shown for several
functional aggregates that strict control mechanisms regulating aggregation are in place. These mechanisms involve not only the quality control system of chaperones and proteases described above, but also genetic and evolutionary strategies to reduce aggregation when inappropriate. The aggregation-prone proteins are under strict transcription, translation and degradation control to make sure that the levels of these proteins are low. Furthermore, gatekeepers play a regulatory role in the assembly process of the major curli subunit CsgA and reduce the potential cytotoxicity of functional amyloids (Wang et al., 2010). It has been suggested that the differential control of aggregation-prone proteins may be a part of a general regulatory framework that not only minimizes unwanted aggregation but also keeps functional aggregation in check (Gsponer & Babu, 2012).

**Implications for antimicrobial strategies**

The knowledge about misfolding, aggregation and its toxicity could be used towards the discovery of new antimicrobials with a completely novel mode of action. For example the caseinolytic proteases (CLps) in various pathogenic bacteria could serve as possible drug targets. The presence of ClpC, ClpP and ClpX was shown to be essential for the viability of *B. subtilis* in various stress conditions. Moreover, mutants lacking any of these ATPases tend to accumulate misfolded proteins as large aggregates (even in non-stress conditions), finally leading to cell death (Kruger et al., 2000). Other independent studies showed that the depletion of ClpX results in attenuated colonization and virulence of *Staphylococcus aureus* and a rapid cell death of *Streptococcus pneumoniae*, without obvious morphological changes (Frees et al., 2003). One of the most recently discovered classes of antibiotics, the acyldepsipeptides (ADEPs), target the proteolytic machinery – more specifically the core unit of the ATP-dependent protease complex, ClpP, and cause uncontrolled proteolysis. ADEPs disturb ClpP activity and change its substrate specificity, hence turning this peptidase into a harmful protease (Brötz-Oesterhelt et al., 2005). Another recent discovery, the IXP1 cyclic peptide, inhibits the degradation of ClpXP substrates and has antibacterial activity towards *Caulobacter crescentus* which requires ClpXP activity for its viability (Cheng et al., 2007). The exact mechanism of action still requires elucidation but primary data revealed that IXP1 is a non-competitive inhibitor of ClpXP (Cheng et al., 2007). Another very interesting attempt to use the protein degradation machinery as an antibacterial target uses oxathiazol-2-one compounds, which kill non-replicating *Mycobacterium tuberculosis* and act as selective suicide-substrate inhibitors of the *M. tuberculosis* proteasome by cyclocondonating its active site threonine (Lin et al., 2009). The short, proline-rich antimicrobial peptides isolated from insects (such as pyrrhocoricin, drosocin and apidecin) were shown to interact with the 70 kDa bacterial heat-shock protein DnaK and this has been directly correlated with their antimicrobial activity (Kragol et al., 2001). The pharmaceutical potential of the proline-rich antimicrobial peptides lies in the fact that they show good selectivity and do not interact with human Hsp70. Aminoglycoside antibiotics, in particular streptomycin and kanamycin (Maisonneuve et al., 2008a), were shown to target the ribosomes, resulting in translational errors (Ling et al., 2012). The rapid bactericidal effect of aminoglycosides is the result of oxidative stress following the appearance of mistranslated proteins, which damages DNA, proteins and membranes. Interestingly, further research into the mechanism of resistance to aminoglycosides revealed that streptomycin causes transient protein aggregation in *E. coli* and unveiled the critical role of alkyl hydroperoxide reductase in protecting bacteria from streptomycin-induced aggregation (Ling et al., 2012). Work on protein aggregation in bacteria following streptomycin treatment showed that chaperones IbpA, IbpB, ClpB and DnaJ are critical for regulating the clearance of aggregated proteins that result from drug-induced erroneous translation. Furthermore, treating *E. coli* cells with streptomycin led to aggregation of metabolic proteins and an increase in the level of protein carbonylation, which is a marker for irreversible protein oxidation. The accumulation of such non-degradable carbonylated proteins in an aggregate state contributes to the increase in carbonyl content observed during bacterial senescence (Dukan & Nyström, 1998; Maisonneuve et al., 2008b). An extensive proteome analysis of oxidized protein upon streptomycin treatment showed that streptomycin-resistant strains overexpress peroxiredoxin AhpCF, a primary scavenger of free radicals (Ling et al., 2012). The overexpression of AhpF in *E. coli* improved the bacterial fitness in the presence of aminoglycosides; therefore inhibiting AhpF could be a good strategy to target resistant strains. Inducing aggregation by infectious prion-like sequence could be another interesting method to manipulate the proteolytic machinery in bacterial cells, given that it was shown that protofibrils may promote their own accumulation by inhibiting proteasomal degradation (Lashuel & Lansbury, 2006). An example of such induced toxicity was given by Garrity et al. (2010), who successfully transferred the Sup35NM prion moiety into *E. coli*, resulting in toxic aggregate formation. Furthermore, it was shown that synthetic peptides derived from the sequence of highly aggregation-prone regions of IBs were able to seed and facilitate incorporation of globular proteins into amyloid structures in healthy *E. coli* (Wang, 2008). Thus, inducing intracellular aggregation or targeting essential for survival proteins, and stimulating their aggregation, could be another promising way to fight pathogens.

**Conclusions**

Although protein aggregation was first discovered as a pathogenic mechanism in mammalian cells, the molecular machinery in the cell to combat misfolding and aggregation, the so-called proteostasis network, turns out to
contain many similarities between eukaryotic and prokaryotic cells. This raises an interesting possibility that the same basic mechanism that can cause neuronal death in e.g., Alzheimer’s disease can be harnessed to combat bacterial infection, provided chemicals can be identified that specifically cause proteostatic collapse in bacteria, but not in the mammalian cells of the host. Moreover, the interference in aggregation of bacterial toxins or adhesins may prevent colonization and pathogenicity of some bacterial species, providing prophylaxis against many difficult-to-treat infections. The data we reviewed here suggest this promising approach holds significant potential that will hopefully materialize into novel therapeutic options to treat bacterial infection in the near future.

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References

Ami, D., Natalelo, A., Schultz, T., Gatti-Lafranconi, P., Lotti, M., Doglia, S. M. & de Marco, A. (2009). Effects of recombinant protein misfolding and aggregation on bacterial membranes. Biochim Biophys Acta 1794, 263–269.

Amijee, H., Madine, J., Middleton, D. A. & Doig, A. J. (2009). Inhibitors of protein aggregation and toxicity. Biochem Soc Trans 37, 692–696.

Balch, W. E., Morimoto, R. I., Dillin, A. & Kelly, J. W. (2008). Adapting proteostasis for disease intervention. Science 319, 916–919.

Balgerrie, A., Dos Reis, S., Ritter, C., Chaignepain, S., Courlay-Salin, B., Forge, V., Bathany, K., Lascu, I., Schmitter, J. M. & other authors (2003). Domain organization and structure–function relationship of the HET-s prion protein of Podospora anserina. EMBO J 22, 2071–2081.

Beerten, J., Schymkowitz, J. & Rousseau, F. (2012). Aggregation prone regions and gatekeeping residues in protein sequences. Curr Top Med Chem 12, 2470–2478.

Bence, N. F., Sampat, R. M. & Kopito, R. R. (2001). Impairment of the ubiquitin–proteasome system by protein aggregation. Science 292, 1552–1555.

Benilova, I., Karran, E. & De Strooper, B. (2012). The toxic Aβ oligomer and Alzheimer’s disease: an emperor in need of clothes. Nat Neurosci 15, 349–357.

Berge, A., Kihlberg, B.-M., Sjöholm, A. G. & Björck, L. (1997). Streptococcal protein H forms soluble complement-activating complexes with IgG, but inhibits complement activation by IgG-coated bacterial protein. J Biol Chem 272, 20774–20781.

Bieler, S., Estrada, L., Lagos, R., Baeza, M., Castilla, J. & Soto, C. (2005). Amyloid formation modulates the biological activity of a bacterial protein. J Biol Chem 280, 26880–26885.

Brötz-Oesterhelt, H., Beyer, D., Kroll, H.-P., Enderrmann, R., Ladel, C., Schroeder, W., Hinzen, B., Raddatz, S., Paulsen, H. & other authors (2005). Dysregulation of bacterial proteolytic machinery by a new class of antibiotics. Nat Med 11, 1082–1087.

Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formiglì, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C. M. & Stefani, M. (2002). Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 416, 507–511.

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

Carrió, M. M. & Villaverde, A. (2003). Role of molecular chaperones in inclusion body formation. FEBS Lett 537, 215–221.

Carrió, M. M., Cubarsi, R. & Villaverde, A. (2000). Fine architecture of bacterial inclusion bodies. FEBS Lett 471, 7–11.

Carrió, M., González-Montalbán, N., Vera, A., Villaverde, A. & Ventura, S. (2005). Amyloid-like properties of bacterial inclusion bodies. J Mol Biol 347, 1025–1037.

Castillo, V., Graña-Montes, R. & Ventura, S. (2011). The aggregation properties of Escherichia coli proteins associated with their cellular abundance. Biotechnol J 6, 752–760.

Chaussée, M. S., Phillips, E. R. & Ferretti, J. J. (1997). Temporal production of streptococcal erythrogenic toxin B (streptococcal cysteine proteinase) in response to nutrient depletion. Infect Immun 65, 1956–1959.

Cheng, L., Naumann, T. A., Horswill, A. R., Hong, S.-J., Venters, B. J., Tomsho, J. W., Benkovic, S. J. & Keiler, K. C. (2007). Discovery of antibacterial cyclic peptides that inhibit the ClpXP protease. Protein Sci 16, 1535–1542.

Chiti, F. & Dobson, C. M. (2009). Amyloid formation by globular proteins under native conditions. Nat Chem Biol 5, 15–22.

Choi, Y.-G., Kim, J.-I., Lee, H.-P., Jin, J.-K., Choi, E.-K., Carp, R. I. & Kim, Y.-S. (2000). Induction of heme oxygenase-1 in the brains of scrapie-infected mice. Neurosci Lett 289, 173–176.

de Groot, N. S. & Ventura, S. (2010). Protein aggregation profile of the bacterial cytosol. PLoS ONE 5, e9383.

de Groot, N. S., Sabate, R. & Ventura, S. (2009). Amyloids in bacterial inclusion bodies. Trends Biochem Sci 34, 408–416.

Demuro, A., Mina, E., Kayed, R., Milton, S. C., Parker, I. & Glabe, C. G. (2005). Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. J Biol Chem 280, 17294–17300.

Dominguez, D. C. (2004). Calcium signalling in bacteria. Mol Microbiol 54, 291–297.

Dougan, D. A., Mogk, A. & Bukau, B. (2002). Protein folding and degradation in bacteria: to degrade or not to degrade? That is the question. Cell Mol Life Sci 59, 1607–1616.

DuBay, K. F., Pawar, A. P., Chiti, F., Zurdo, J., Dobson, C. M. & Vendruscolo, M. (2004). Prediction of the absolute aggregation rates of amyloidogenic polypeptide chains. J Mol Biol 341, 1317–1326.

Dukan, S. & Nyström, T. (1998). Bacterial senescence: stasis results in increased and differential oxidation of cytoplasmic proteins leading to developmental induction of the heat shock regulon. Genes Dev 12, 3431–3441.

Fink, A. L. (1998). Protein aggregation: folding aggregates, inclusion bodies and amyloid. Folding Des 3, R9–R23.

Fowler, D. M., Koulav, A. V., Balch, W. E. & Kelly, J. W. (2007). Functional amyloid – from bacteria to humans. Trends Biochem Sci 32, 217–224.

Frees, D., Qazi, S. N. A., Hill, P. J. & Ingmer, H. (2003). Alternative regulation of cysteine proteinase (cysteine proteinase) in response to nutrient depletion. Biochim Biophys Acta 1642, 291–297.

Fricke, I. M., Mo¨ rgelin, M. & Bjo¨ rck, L. (2000). Alternative regulation of cysteine proteinase in response to nutrient depletion. Biochim Biophys Acta 1510, 169–178.

Frint, C. L., Ackert, K. D., Davis, S. R., Gross, W., Davis, S. R., Ackert, K. D. & Gross, W. (2003). Staphylococcus aureus and Streptococcus pyogenes: diverse mechanisms of bacterial virulence. Annu Rev Genet 37, 343–370.
García-Fruitós, E., González-Montalbán, N., Morell, M., Vera, A., Ferraz, R. M., Aris, A., Ventura, S. & Villaverde, A. (2005). Aggregation as bacterial inclusion bodies does not imply inactivation of enzymes and fluorescent proteins. *Microb Cell Fact* 4, 27.

Garland, W. J. & Buckley, J. T. (1988). The cytolytic toxin aerolysin must aggregate to disrupt erythrocytes, and aggregation is stimulated by human glycoporphin. * Infect Immun* 56, 1249–1253.

Garrity, S. J., Sivanathan, V., Dong, J., Lindquist, S. & Hochschild, A. (2010). Conversion of a yeast prion protein to an infectious form in bacteria. * Proc Natl Acad Sci U S A* 107, 10596–10601.

Glaube, C. G. (2006). Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. * Neurobiol Aging* 27, 570–575.

Green, J. D., Kreplak, L., Goldsbury, C., Li Blatter, X., Stolz, M., Cooper, G. S., Seelig, A., Kistler, J. & Aebl, U. (2004). Atomic force microscopy reveals defects within mica supported lipid bilayers induced by the amyloidogenic human amylin peptide. *J Mol Biol* 342, 877–887.

Gsponer, J. & Babu, M. M. (2012). Cellular strategies for regulating functional and nonfunctional protein aggregation. * Cell Rep* 2, 1425–1437.

Guentchev, M., Voigtländer, T., Haberler, C., Groschup, M. H. & Budka, H. (2000). Evidence for oxidative stress in experimental prion disease. *Neurobiol Dis* 7, 270–273.

Guisbert, E., Herman, C., Lu, C. Z. & Gross, C. A. (2004). A chaperone network controls the heat shock response in *E. coli*. *Genes Dev* 18, 2812–2821.

Hartl, F. U., Bracher, A. & Hayer-Hartl, M. (2011). Molecular chaperones in protein folding and proteostasis. *Nature* 475, 324–332.

Herbaud, M.-L., Guiseppi, A., Denizot, F., Haiech, J. & Kilhoffer, M.-C. (2008). Calcium signaling in *Bacillus subtilis*. *Biochim Biophys Acta* 1448, 212–226.

Hirakura, Y., Azimov, R., Azimova, R. & Kagan, B. L. (2000). Polyglutamine-induced ion channels: a possible mechanism for the neurotoxicity of Huntington and other CAG repeat diseases. *J Neurosci Res* 60, 490–494.

Hotze, E. M., Heuck, A. P., Czajkowsky, D. M., Shao, Z., Johnson, A. E. & Tweten, R. K. (2002). Monomer–monomer interactions drive the prepore to pore conversion of a β-barrel-forming cholesterol-dependent cytoplytic. *J Biol Chem* 277, 11597–11605.

Hyun, D.-H., Lee, M., Hattori, N., Kubo, S.-I., Mizuno, Y., Halliwell, B. & Jenner, P. (2002). Effect of wild-type or mutant Parkin on oxidative damage, nitric oxide, antioxidiant defenses, and the proteasome. *J Biol Chem* 277, 28572–28577.

Jahn, T. R. & Radford, S. E. (2005). The Yin and Yang of protein folding. *FEBS J* 272, 5962–5970.

Kagan, B. L., Hirakura, Y., Azimov, R. & Azimova, R. (2001). The channel hypothesis of Huntington’s disease. *Brain Res Bull* 56, 281–284.

Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W. & Glabe, C. G. (2003). Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 300, 486–489.

Kragol, G., Lovas, S., Varadi, G., Condie, B. A., Hoffmann, R. & Otvos, L, Jr (2001). The antibacterial peptide pyrrhocoricin inhibits the ATPase actions of DnaK and prevents chaperone-assisted protein folding. *Biochemistry* 40, 3016–3026.

Kress, W., Maglica, Z. & Weber-Ban, E. (2009). Clp chaperone-proteases: structure and function. *Res Microbiol* 160, 618–628.

Krüger, E., Witt, E., Ohlmeier, S., Hanschke, R. & Hecker, M. (2000). The Clp proteases of *Bacillus subtilis* are directly involved in degradation of misfolded proteins. *J Bacteriol* 182, 3259–3265.

Lashuel, H. A. & Lansbury, P. T., Jr (2006). Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins? *Q Rev Biophys* 39, 167–201.

Liberek, K., Lewandowska, A. & Zietkiewicz, S. (2008). Chaperones in control of protein disaggregation. *EMBO J* 27, 328–335.

Lin, G., Li, D., de Carvalho, L. P. S., Deng, H., Tao, H., Vogt, G., Wu, K., Schneider, J., Chidawanyika, T. & other authors (2009). Inhibitors selective for mycobacterial versus human proteasomes. *Nature* 461, 621–626.

Lindner, A. B., Madden, R., Demarez, A., Stewart, E. J. & Taddei, F. (2008). Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. *Proc Natl Acad Sci U S A* 105, 3076–3081.

Ling, J., Cho, C., Guo, L. T., Aerni, H. R., Rinehart, J. & Söll, D. (2012). Protein aggregation caused by aminoglycoside action is prevented by a hydrogen peroxide scavenger. *Mol Cell* 48, 713–722.

Maisonneuve, E., Fraysse, L., Moinier, D. & Dukan, S. (2008a). Existence of abnormal protein aggregates in healthy *Escherichia coli* cells. *J Bacteriol* 190, 887–893.

Maisonneuve, E., Fraysse, L., Lignon, S., Capron, L. & Dukan, S. (2008b). Carbonylated proteins are detectable only in a degradation-resistant aggregate state in *Escherichia coli*. *J Bacteriol* 190, 6609–6614.

Makin, O. S., Atkins, E., Sikorski, P., Johansson, J. & Serpell, L. C. (2005). Molecular basis for amyloid fibril formation and stability. *Proc Natl Acad Sci U S A* 102, 315–320.

Markoskiss, K. A. & Kurganov, B. I. (2004). Protein folding, misfolding, and aggregation. Formation of inclusion bodies and aggresomes. *Biochemistry (Moscow)* 69, 971–984.

Martínez-Alonso, M., González-Montalbán, N., García-Fruitós, E. & Villaverde, A. (2009). Learning about protein solubility from bacterial inclusion bodies. *Microb Cell Fact* 8, 4.

Maurer-Stroh, S., Debulpae, M., Kuemmerer, N., Lopez de la Paz, M., Martins, I. C., Reumers, J., Morris, K. L., Copland, A., Serpell, L. & other authors (2010). Exploring the sequence determinants of amyloid structure using position-specific scoring matrices. *Nat Methods* 7, 237–242.

Menozzi, F. D., Boucher, P. E., Riveau, G., Gantiez, C. & Locht, C. (1994). Surface-associated filamentous hemagglutinin induces auto-agglutination of Bordetella pertussis. * Infect Immun* 62, 4261–4269.

Menozzi, F. D., Rouse, J. H., Alvi, M., Laude-Sparch, M., Muller, J., Bischoff, R., Brennan, M. J. & Locht, C. (1996). Identification of a heparin-binding hemagglutinin present in mycobacteria. *J Exp Med* 184, 993–1001.

Merino, N., Toledo-Arana, A., Vergara-Irigaray, M., Valle, J., Solano, C., Calvo, E., Lopez, J. A., Foster, T. J., Penadés, J. R. & Lasa, I. (2009). Protein A-mediated multicellular behavior in *Staphylococcus aureus*. *J Bacteriol* 191, 832–843.

Milhavet, O. & Lehmann, S. (2002). Oxidative stress and the prion protein in transmissible spongiform encephalopathies. *Brain Res Rev* 38, 328–339.

Mogk, A., Mayer, M. P. & Deuerling, E. (2002). Mechanisms of protein folding: molecular chaperones and their application in biotechnology. *ChemBioChem* 3, 807–814.

Monoi, H. (1995). New tubular single-stranded helix of poly-γ-amino acids suggested by molecular mechanics calculations: I. Homopolypeptides in isolated environments. *Biophys J* 69, 1130–1141.

Morgan, G. J., Giannini, S., Hounslow, A. M., Craven, C. J., Zerovnik, E., Turk, V., Waltho, J. P. & Staniforth, R. A. (2008). Glucocorticoids inhibit amyloid fibrillogenesis in vitro: an in vitro study of the native α-helix from the amyloid fibrils of a mixed α/β protein. *J Mol Biol* 375, 487–498.

Liberek, K., Lewandowska, A. & Zietkiewicz, S. (2008). Chaperones in control of protein disaggregation. *EMBO J* 27, 328–335.
Muchowski, P. J. & Wacker, J. L. (2005). Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci* **6**, 11–22.

Nelson, R., Sawaya, M. R., Balbirnie, M., Madsen, A. O., Riekel, C., Grothe, R. & Eisenberg, D. (2005). Structure of the cross-β spine of amyloid-like fibrils. *Nature* **435**, 773–778.

Okumura, S., Saitoh, H., Ishikawa, T., Inouye, K. & Mizuki, E. (2011). Mode of action of parasporin-4, a cytoidal protein from *Bacillus thuringiensis*. *Biochim Biophys Acta* **1808**, 1476–1482.

Olzscha, H., Schermann, S. M., Woerner, A. C., Pinkert, S., Hecht, M. H., Tartaglia, G. G., Vendruscolo, M., Hayer-Hartl, M., Hartl, F. U. & Vabulas, R. M. (2011). Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. *Cell* **144**, 67–78.

Ono, K., Takahashi, R., Ikeda, T. & Yamada, M. (2012). Cross-seeding effects of amyloid β-protein and α-synuclein. *J Neurochem* **122**, 883–890.

Otzen, D. & Nielsen, P. H. (2008). We find them here, we find them there: functional bacterial amyloid. *Cell Mol Life Sci* **65**, 910–927.

Quist, A., Doudevski, I., Lin, H., Azimova, R., Ng, D., Frangione, B., Kagan, B., Ghiso, J. & Lal, R. (2005). Amyloid ion channels: a common structural link for protein-misfolding disease. *Proc Natl Acad Sci U S A* **102**, 10427–10432.

Reumers, J., Maurer-Stroh, S., Schymkowitz, J. & Rousseau, F. (2009). Protein sequences encode safeguards against aggregation. *Hum Mutat* **30**, 431–437.

Robertson, G. T., Ng, W.-L., Gilmour, R. & Winkler, M. E. (2003). Essentiality of clpX, but not clpP, clpL, clpC, or clpE, in *Streptococcus pneumoniae* R6. *J Bacteriol* **185**, 2961–2966.

Romero, D., Aguilar, C., Losick, R. & Kolter, R. (2010). Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. *Proc Natl Acad Sci U S A* **107**, 2230–2234.

Rousseau, F., Schymkowitz, J. & Serrano, L. (2006a). Protein aggregation and amyloidosis: confusion of the kinds? *Curr Opin Struct Biol* **16**, 118–126.

Rousseau, F., Serrano, L. & Schymkowitz, J. W. (2006b). How evolutionary pressure against protein aggregation shaped chaperone specificity. *J Mol Biol* **355**, 1037–1047.

Sarell, C. J., Woods, L. A., Su, Y., Deboulouchina, G. T., Ashcroft, A. E., Griffin, R. G., Stockley, P. G. & Radford, S. E. (2013). Expanding the repertoire of amyloid polymers by co-polymerization of related protein precursors. *J Biol Chem* **288**, 7327–7337.

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

Schlieker, C., Bukau, B. & Mogk, A. (2002). Prevention and reversion of protein aggregation by molecular chaperones in the *E. coli* cytosol: implications for their applicability in biotechnology. *J Biotechnol* **96**, 13–21.

Steffan, J. S., Kazantsev, A., Spasic-Boskovic, O., Greenwald, M., Zhu, Y. Z., Gohler, H., Wanker, E. E., Bates, G. P., Housman, D. E. & Thompson, L. M. (2000). The Huntington’s disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc Natl Acad Sci U S A* **97**, 6763–6768.

Stöver, A. G. & Driks, A. (1999). Secretion, localization, and antibacterial activity of TasA, a *Bacillus subtilis* spore-associated protein. *J Bacteriol* **181**, 1664–1672.

Su, C.-Y., Chong, K.-Y., Edelstein, K., Lille, S., Khardori, R. & Lai, C.-C. (1999). Constitutive hsp70 attenuates hydrogen peroxide-induced membrane lipid peroxidation. *Biochem Biophys Res Commun* **265**, 279–284.

Tartaglia, G. G. & Vendruscolo, M. (2009). Correlation between mRNA expression levels and protein aggregation propensities in subcellular localisations. *Mol Biosyst* **5**, 1873–1876.

Tyedmers, J., Mogk, A. & Bukau, B. (2010). Cellular strategies for controlling protein aggregation. *Nat Rev Mol Cell Biol* **11**, 777–788.

Ventura, S. & Villaverde, A. (2006). Protein quality in bacterial inclusion bodies. *Trends Biotechnol* **24**, 179–185.

Vera, A., Aris, A., Carrió, M., González-Montalbán, N. & Villaverde, A. (2005). Lon and ClpP proteases participate in the physiological disintegration of bacterial inclusion bodies. *J Biotechnol* **119**, 163–171.

Villa, R., Lotti, M. & Gatti-Lafranconi, P. (2009). Components of the *E. coli* envelope are affected by and can react to protein over-production in the cytoplasm. *Microb Cell Fact* **8**, 32.

Wang, X., Smith, D. R., Jones, J. W. & Chapman, M. R. (2007). In vitro polymerization of a functional *Escherichia coli* amyloid protein. *J Biol Chem* **282**, 3713–3719.

Wang, L., Maji, S. K., Sawaya, M. R., Eisenberg, D. & Riek, R. (2008). Bacterial inclusion bodies contain amyloid-like structure. *PLoS Biol* **6**, e195.

Wang, X., Zhou, Y., Ren, J. J., Hammer, N. D. & Chapman, M. R. (2010). Gatekeeper residues in the major curlin subunit modulate bacterial amyloid fiber biogenesis. *Proc Natl Acad Sci U S A* **107**, 163–168.

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