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Amyloidogenicity as a driving force for the formation of functional oligomers

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Abstract

Insoluble amyloid fibrils formed by self-assembly of amyloidogenic regions of proteins have a cross- β -structure. In this work, by using targeted molecular dynamics and rigid body simulation, we demonstrate that if a protein consists of an amyloidogenic region and a globular domain(s) and if the linker between them is short enough, such molecules cannot assemble into amyloid fibrils, instead, they oligomers with a defined and limited number of β -strands in the cross- β core. We show that this blockage of the amyloid growth is due to the steric repulsion of globular structures linked to amyloidogenic regions. Furthermore, we establish the relationship between the linker length and the number of monomers in such nanoparticles. We hypothesise that such oligomerisation can be a yet unrecognised way to form natural protein complexes involved in biological processes. Our results can also be used in protein engineering for designing soluble nanoparticles carrying different functional domains.

KEYWORDS: Amyloids, targeted molecular dynamics, rigid body simulation, functional nanoparticles.

1. Introduction

Amyloid fibrils are the subject of particular interest due to their association with a number of human diseases. Despite considerable importance, until recently, the structural arrangement of amyloid fibrils was poorly understood compared with soluble proteins. This was due to the fact that conventional experimental methods (X-ray crystallography and NMR in solution) capable of determining high-resolution structure, cannot be used because of the insolubility of fibrils. The recent progress in understanding the amyloid structure has stemmed mostly from the application of experimental techniques such as solid-state nuclear magnetic resonance, cryo-electron microscopy, and scanning transmission electron microscopy mass measurements (Nelson and Eisenberg 2006; Steven et al. 2016). The majority of the naturally-occurring amyloid structures have been shown to have a cross- β structure with parallel and in-register β -strands (Colvin et al. 2016; Gorkovskiy et al. 2014; Groveman et al. 2014; Helmus et al. 2011; Kajava et al. 2004; Kajava, Baxa, and Steven 2010; Luckgei et al. 2013; Murray et al. 2017; Rodriguez et al. 2015; Vilar et al. 2008; Wasmer et al. 2008; Weirich et al. 2016) (Fig. 1a). The formation of the parallel and in-register structures from the amyloidogenic polypeptides is spontaneous and leads to the stable cross- β fibrils (Colvin et al. 2016; Gorkovskiy et al. 2014; Groveman et al. 2014; Helmus et al. 2011; Kajava et al. 2004, 2010; Luckgei et al. 2013; Luhrs et al. 2005; Vilar et al. 2008; Weirich et al. 2016), suggesting that the interaction between the β -structural subunits of amyloids is energetically favourable. These interactions are strong enough to bring together large proteins containing, in addition to the amyloidogenic region (AR), globular domains and long unfolded linkers, as observed, for example, in Rip1/Rip3, huntingtin, and TAR-DNA binding protein, Hets, Sup35p, Ure2p and Prp (Baxa et al. 2006, 2007; Chen et al. 2010; Groveman et al. 2014; Helmus et al. 2011; Li et al. 2012; Pfefferkorn, McGlinchey, and Lee 2010; Shen et al. 2016; Shewmaker et al. 2009; Wasmer et al. 2009) (Fig. 1b). Usually, in these fibrils, the unfolded linker connecting the amyloidogenic regions (AR) and globular domain is long (more than 50 residues), since AR location in close proximity to the globular domain along the chain would cause steric repulsion and prevent the formation of amyloid structures (Kajava et al. 2004) (Fig. 1c). Recently, by using molecular and mesoscopic modelling, we were able to establish a relationship between the size of the globular domains and the length of the shortest possible linker that still allows the formation

of the infinite amyloid fibrils having a cross- β structure with parallel and in-register β -strands (Azizyan et al., 2018). This relationship was confirmed experimentally (Wang et al. 2019). The objective of the present work was to study the self-assembly of ARs when the linker between the AR and the globular domain is shorter than the one allowing the formation of the infinite fibrils.

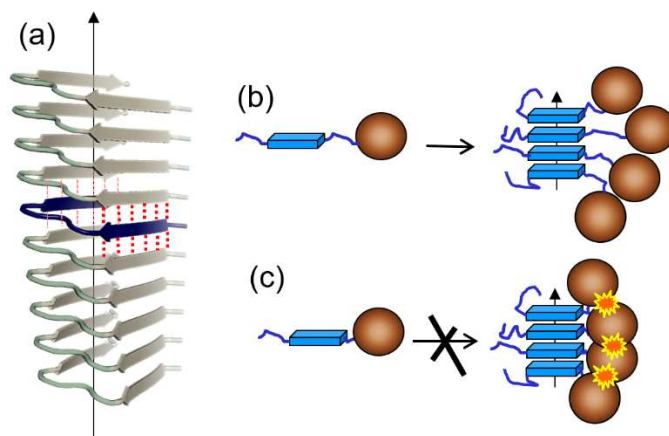


Fig. 1. Structural arrangement of amyloid fibril and two possible scenarios of amyloid structure formation in the presence of flanking globular domains. **a** A typical structural arrangement of amyloid fibrils. The β -arch is stacked in a parallel and in-register manner into fibrillar ‘ β -arcade’ structure. Two strands of the β -arch are integrated into two different β -sheets. **b** Stack of ARs (as boxes of blue colour) connected with globular domains (spheres of brown colour) by long linkers. These molecules can form infinite amyloid fibrils. The fibre axis is indicated by a thin vertical arrow. **c** Stack of ARs connected with globular domains by short linkers. The stacking of ARs is hampered by the steric repulsion of globular domains.

2. Materials and methods

Coarse-grained targeted molecular dynamics. We used the CG-TMD approach implemented with the GROMACS package (version 4.6.7) (Hess et al. 2008; Van Der Spoel et al. 2005) installed on a multi-processor Xeon based workstation with an SSE4.1 CPU acceleration set. To evaluate the steric tensions and overall stereochemistry of the oligomers obtained during the coarse-grained TMD, we converted them back to the all-atom structures by using the “Going backward” tool (Wassenaar et al. 2014). Then, we applied an energy minimisation procedure using the steepest descent algorithm of the GROMACS package version 4.6.7 (Hess et al. 2008; Van Der Spoel et al. 2005) and evaluated steric tension and overall stereochemistry of the structures by

using the PROCHECK package (Laskowski et al. 1993). For further details, see Supplementary Data.

Rigid Body Simulation. As a simplified mesoscopic modelling strategy, we used the Low Poly 3D models and Rigid Body Simulation implemented in Blender software package³⁷. For additional information, see <https://cg3dartist.wixsite.com/amyloid>. The final structures were checked for the absence of intersections between the elements of models with Mesh Analysis tool of Blender. All other geometrical parameters and Rigid Body World setting were the same as in the previous work³⁶. For further details, see Supplementary Data.

3. Results

3.1 Modelling of self-assembly of a hybrid protein containing AR and typical globular domain by using full-atom and coarse-grained targeted molecular dynamics. To analyse the constraints on amyloid formation imposed by steric exclusion of globular domains, we used a hybrid protein containing an amyloid-forming fragment of A β peptide (17-42) and Green Fluorescent Protein (GFP) as a globular domain. This choice is explained by the fact that on the one hand, the A β -fragment is the most studied amyloid-forming peptide and, on the other hand, the GFP globular domain is the most frequently fused to different amyloid-forming domains (Fox et al. 2010; Ochiishi et al. 2016; Waldo et al. 1999; Wurth, Guimard, and Hecht 2002). Besides, GFP, with its 238 residues, represents a typical globular domain being close to the average size of the globular domain (190 residues in MODBASE) (Pieper et al. 2014). The structure of the A β protofibril was taken from the PDB entry 2BEG (Luhrs et al. 2005). In this amyloid structure, each polypeptide chain is folded into β -arches and stacked in parallel and in-register manner (Fig. 1a). The 3D structure of GFP was taken from PDB entry 1GFL (Yang, Moss, and Phillips 1996). In our model, the linker between these domains has a Ser-Pro-rich sequence, which has the potential of flexible and intrinsically unfolded conformation (Fig. 2a and Supplementary Fig.1). In particular, 1, 2, 3, 4, 5, and 6-residue linkers consist of Ser, Ser-Ser, Pro-Ser-Ser, Ser-Pro-Ser-Ser, Ser-Ser-Pro-Ser-Ser, Pro-Ser-Ser-Pro-Ser-Ser, respectively.

Molecular modelling showed that A β -GFP with linkers shorter than 7 residues can still form the amyloid cross- β structures; however, these complexes represent oligomers with a limited number of the monomers because of the steric repulsion of the globular domains. We were able to establish the relationship between the number of subunits in the oligomers and the length of the linker. For this analysis, we modelled the self-assembly of A β (17-42)-linker-GFP molecules by using targeted molecular dynamics (TMD) (Schlitter et al. 1993). If initial and target conformations are known, then the TMD simulation is a suitable method to predict the transition pathways by continuously diminishing the RMS distance value between these conformations. In our case, the initial state of AR is an unfolded conformation, and the final structure is a β -arch within the β -arcade (Luhrs et al. 2005) (Fig. 1a). Concerning the other parts of the hybrid molecule, the GFP globular domain was kept in its initial crystal structure, and linkers of a given length were free of constraints and flexible. The monomers were added to the growing complexes until they started to have steric tensions. Our TMD simulations, made by using the full-atom system with and without explicit water, show that this system is calculation expensive. Therefore, we turned our attention to the TMD applied to coarse-grained (CG) molecular models (Monticelli et al. 2008; Tozzini, Rocchia, and McCammon 2006). In CG models, the reduced number of degrees of freedom and the use of smoother interaction potentials allow for longer time steps, resulting in a significant increase in the calculation speed (Dror et al. 2010). Our tests showed that the CG-TMD approach implemented with the GROMACS package fits our system well. An addition of a monomer to the β -arcade target requires about 18 hours of CPU time on a multi-processor Xeon based workstation with an SSE4.1 CPU acceleration set. To evaluate the steric tensions and overall stereochemistry of the oligomers obtained during the coarse-grained TMD, we converted them back to the all-atom structures by using the “Going backward” tool (Wassenaar et al. 2014). The oligomer structures with disallowed atomic contacts or covalent structure geometry were rejected. (For details see Supplementary Data).

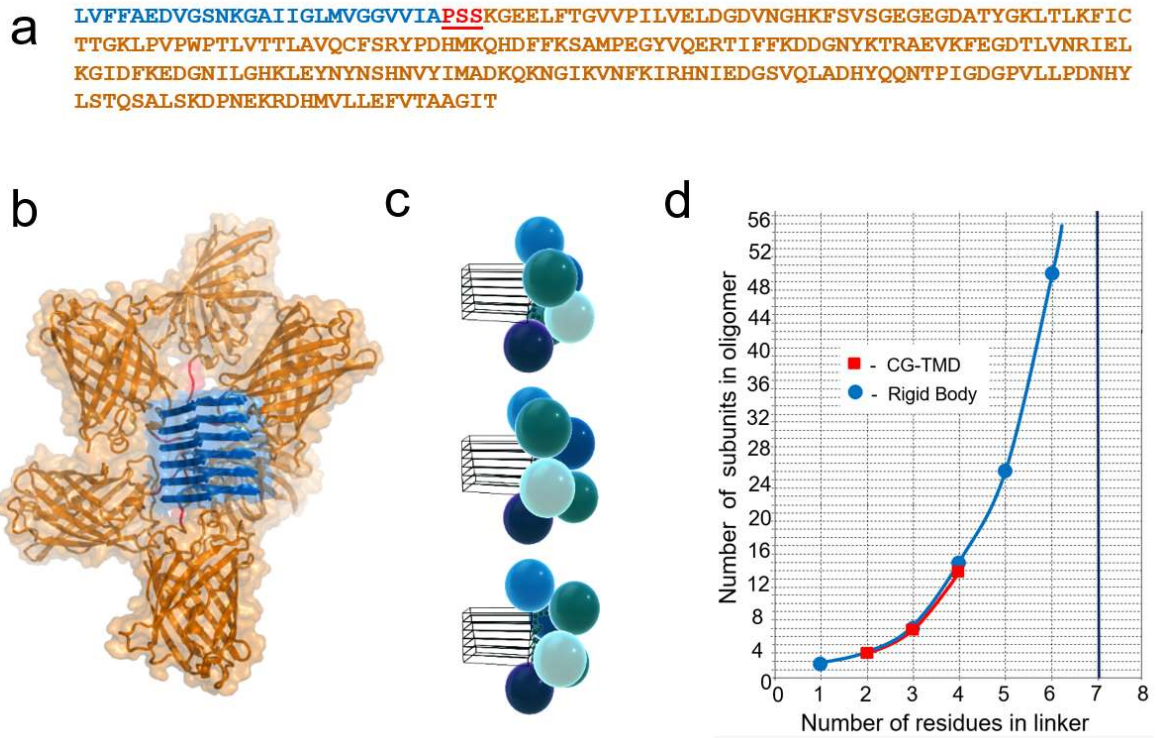


Fig. 2. Models of an amyloid-like oligomer formed by hybrid molecule $A\beta(17-42)$ -linker-GFP with 3 residue linker. **a** Sequence of $A\beta(17-42)$ -linker-GFP monomer with $A\beta(17-42)$ peptide (PDB entry 2BEG) (in blue), a linker (red and underlined) composed of Pro-Ser-Ser sequence (C-terminal Ser comes from the N-terminus of GFP), and GFP domain from *Aequorea victoria* (PDB entry 2Y0G) (in light brown). **b** The all-atom 3D structure of a hexamer containing monomers with 3 residues in the linker obtained by CG MD simulations (picture was generated using VMD package (Humphrey, Dalke, and Schulten 1996)) (regions of the hybrid molecule are coloured similarly on **a** and **b**). **c** Several arrangements of the hexamers obtained in the different runs of the Blender Rigid Body simulation. **d** Dependence of the number of subunits in oligomer on the linker length. The vertical line at the 7-residue value denotes the minimal number of residues in the linker allowing the growth of infinite amyloid fibril. Red squares and blue points correspond to data obtained in CG-TMD and rigid body simulations, correspondingly.

The results of our analysis are shown in Fig. 2b and Fig. 2d. When the linker consists of 1, 2, 3 or 4 residues, the oligomers can have maximum 2, 3, 6 or 13 subunits, correspondingly. The longer the linker, the higher stoichiometry of the oligomer structure, and more calculation time is required to reach the steric limits. Given that $A\beta(17-42)$ -linker-GFP molecules with a 7-residue linker can

form the infinite fibril (Azizyan et al. 2018), we expected a sharp increase in the number of subunits for the molecules with 5 or 6 residue linkers. In this case, the precise estimation of the number of subunits in the oligomer was becoming computationally expensive and would require the other modelling approaches. Thus, our study revealed some limitations of CG-TMD simulations when this method was applied to the larger oligomers.

3.2 Generalisation of the conclusions by using rigid body simulation. The results of CG-TMD described in the previous section can be used to validate the other simplified modelling approaches. At the same time, the simplified approaches can allow extending our conclusion to a more general case, with different linker lengths, shapes, and sizes of the AR and globular domains. As a simplified mesoscopic modelling strategy, we used the Rigid Body Simulation implemented in Blender software package (Anon 2015) (for details see Supplementary Data). The globular part was represented as a sphere with a diameter of 30 Å. All other geometrical parameters and Rigid Body World setting were the same as in the previous work (Azizyan et al. 2018). The obtained dependence between the number of subunits in oligomers and the number of residues in the linkers is shown in Fig. 2d, Fig. 3 and Supplementary Table 1. The comparison of the results derived from the rigid body simulation and CG-TMD showed a good agreement for the linkers with 2, 3, and 4 residues. This comparison allowed us to validate the rigid body simulation results on the CG TMD results. The agreement between the results suggested that the simplified approach could be successfully used for rapid estimations of the larger systems. For example, for the linkers of 5 and 6 residues, the rigid body simulation gave 29 and 69 monomers in the oligomers, respectively (Fig. 3). The simplified method was rapid enough to run several simulations for the same system. It was shown that depending on the initial positions of monomers during the simulation, the stoichiometry of the obtained oligomers can vary (Fig. 3 and Supplementary Table 1). Moreover, even when the number of monomers in the oligomers is the same, the arrangement of the globular parts around the amyloid core can be slightly different (Fig. 2c). This variation can be explained by the local “kinetic traps” presented in such large molecular complexes.

Our modelling was done for GFP, which size is close to the average one of the globular domains (Pieper et al. 2014). At the same time, if the linker length is fixed, the number of subunits in the oligomer should vary depending on the size of the globular structure. To demonstrate this, we extended the rigid body simulations to the oligomers formed by subunits with globular domains

of 40 Å or 50 Å in diameter (Fig 3). For example, in accordance with this estimation, molecules with 6 residue linker and globular domain of 30, 40 or 50 Å can form oligomers of 69, 18 or 12 subunits, correspondingly.

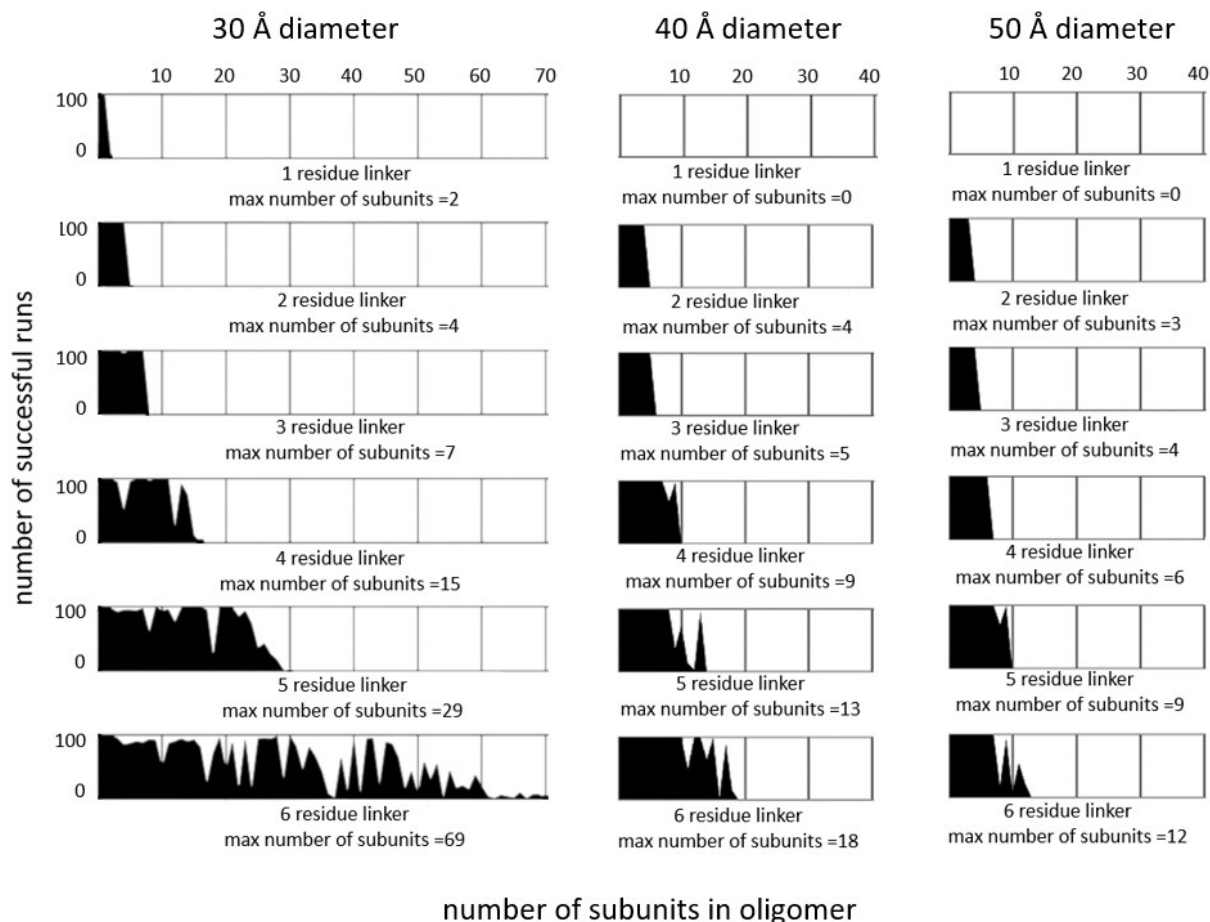


Fig. 3. Dependence between the number of subunits in the cross- β oligomers and the number of residues in the linkers obtained by rigid body simulation. Globular domain was represented by a sphere of either 30, 40 or 50 Å in diameter. Linkers vary from 1 to 6 residues. For a given linker size and number of subunits, we performed 100 rigid body simulations and counted the number of minimized oligomers without steric repulsion.

The rigid body simulation does not take into consideration either a specific amino acid sequence of the linker or its direction in terms of N- and C-terminus. The agreement between the results of the CG TMD and rigid body simulations suggests that our results can be extended to a wide range of linker sequences, regardless of whether the globular domain is N- or C-terminal to the amyloidogenic region. At the same time, the linker sequences should be non-amyloidogenic and have flexible and intrinsically unfolded conformation.

A methodological result of this work is the implementation of CG TMD and Rigid Body simulations as suitable tools to predict the assembly of such large systems containing molecules with amyloid-forming regions linked to globular structures. The modelling protocol established here can be used in future studies. Here, we analysed the most common case of naturally occurring amyloids with the parallel and in-register cross- β structure, but some of the cross- β amyloid fibrils are built by antiparallel stacking of β -arches (Qiang et al. 2012) or short β -solenoids (Wasmer et al. 2008). These fibrils can better tolerate the short linkers of the globular domains and can be analysed by using the approaches presented here. ARs that are flanked by two globular domains on both sides can also be tested.

4. Discussion

Flanking domains of the amyloidogenic regions can affect the formation and the stability of cross- β amyloids. In the most well-studied amyloids, the unfolded linker connecting the amyloidogenic region and globular domain is long (more than 50 residues) (Baxa et al. 2006, 2007; Chen et al. 2010; Groveman et al. 2014; Helmus et al. 2011; Li et al. 2012; Pfefferkorn, McGlinchey, and Lee 2010; Shen et al. 2016; Shewmaker et al. 2009; Wasmer et al. 2009). In this case, the thermal motion of the globular domain and long unfolded flanking region should inhibit the amyloidogenesis by pushing away the amyloidogenic regions due to an excluded volume effect (Rubinshtein and Colby, 2003). Here, we considered proteins with globular structures linked to amyloidogenic regions by short (less than 7 residues) linkers. We showed that the amyloid growth of these proteins is blocked due to the steric repulsion of their globular domains. In comparison with the relatively weak inhibition of the long linkers, which can only shorten the fibrils, the steric effect of proteins with the short linkers is abrupt and strong. As a result, proteins with short linkers form oligomers with a cross- β core of a defined and limited size instead of the fibrils.

In most cases, amyloid fibril formation is nucleation-dependent, displaying a lag phase followed by rapid fibrillogenesis (Ferrone 1999; Xue, Homans, and Radford 2008). Knowledge about the size of the amyloid nucleus may inform us of the size in which the oligomers described here are becoming stable. Nowadays, the question about the exact number of monomers in the nuclei of amyloids is still debated. In the β -arcade, the formation of the first intermolecular H-bonds between two stacked β -arches is the most important step (Kajava et al. 2010), though it is not clear how many additional β -arches are needed to make this β -arcade stable. In other works, it was suggested that the nucleus of amyloid fibrils might consist of 4, 6, and even up to 30 monomers (Nelson et al. 2005, Xue et al. 2008, Sorci et al. 2011). If we consider the highest number of monomers suggested for the nuclei structures, we can conclude that our hybrid molecules form stable oligomers when the linkers are 5 or more residues long (Fig. 3).

Our results suggest that nature can use this type of amyloid-like oligomerisation to form soluble complexes carrying functional domains. A number of protein oligomers, including α -helical coiled coils, triple-helical collagen structures and complexes made from globular domains have been characterised (Steven et al. 2016). The amyloid-like oligomers described in this work have not been observed by the conventional methods of structural biology (such as X-ray crystallography or NMR spectroscopy) yet. This can be explained by the heterogeneity of these superstructures, differing in the number of monomers and in their spatial arrangement around the amyloid-like core (Fig. 2c, Supplementary Table 1), which hampers their atomic-level structural determination. The oligomerisation observed here may also provide structural insight into a growing body of evidence on the phase separation behaviour of proteins with amyloidogenic regions (Alberti et al. 2018). Depending on conditions, several proteins with amyloid-forming regions, such as Sup35p, TDP-43, p53, CPEB3, FUS and others, also have potential to form assemblies with spherical morphologies (Mészáros et al., 2020). These observations put forth questions about the relationship between phase separation and amyloid fibrils. Our results suggest that in some cases, the aggregation state of the amyloidogenic region may be controlled by folding of the neighbouring domains.

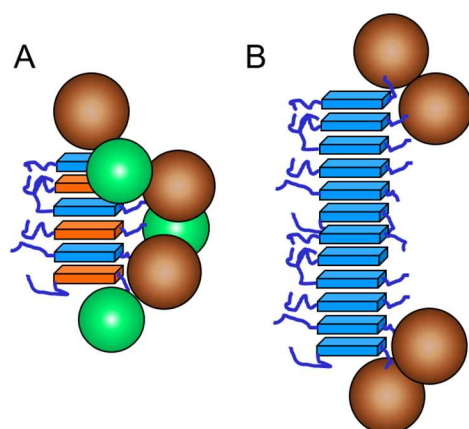


Fig. 4. Schematic representation of some potential applications of amyloid-like oligomeric structures. **A.** Soluble oligomers carrying several different functional domains. **B.** Inhibition of the amyloid fibril growth by addition of hybrid molecules with globular domains.

This proof-of-concept study opens up further opportunities for the fabrication of nanostructures of defined size carrying multiple functional domains (Fig. 4A). These engineered proteins can also be exploited to inhibit amyloidosis, by adding to the amyloidogenic proteins containing a given ARs a hybrid molecule consisting of the same AR linked to a globular domain (Fig. 4B). The amyloid fibril scaffolding is already used in protein engineering to build up functional assemblies (Giraldo 2010). However, in comparison with the insoluble amyloid fibrils, the oligomers may be soluble, suggesting new opportunities in nanobiotechnology.

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Additional information

Detailed methods, an additional table and figures are presented in Supporting Information.

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