Beyond neurodegeneration: engineering amyloids for biocatalysis

Andrea Bartolomé-Nafría, Javier García-Pardo*, Salvador Ventura*

Amyloid fibrils are highly organized protein or peptide aggregates, often characterized by a distinctive supramolecular cross-β-sheet structure. The formation and accumulation of these structures have been traditionally associated with neural or systemic human diseases, such as Alzheimer's disease, Parkinson's disease, type-2 diabetes, or amyotrophic lateral sclerosis (Wei et al., 2017; Wittung-Stafshede, 2023). However, evidence exists that the amyloid fold is also exploited by nature to perform several functional, nonpathogenic roles across all kingdoms of life. For example, amyloids contribute to biofilm formation in bacteria (Peña-Díaz et al., 2024) or are involved in the regulation of transcription and alternative splicing in humans. As a general trend, amyloids display highly rigid structures with high chemical and mechanical stability, which makes them ideal scaffolds for the design of novel nanostructured materials.

The value of amyloids as future bionanomaterials gained significant traction with the discovery of the first catalytically active amyloids (Rufo et al., 2014). Using the active site of carbonic anhydrase as a template, Rufo et al. (2014) designed a first generation of scaffolds built up by seven-residue peptide sequences that intercalated hydrophobic and polar amino acids. The resultant supramolecular assembly featured a nonpolar inner core with polar residues exposed to the solvent. These surface residues are readily available for interaction with substrates and cofactors, enabling the design and development of novel catalytically active nanostructured materials (Rufo et al., 2014).

Harnessing the potential of amyloids for creating novel nanozymes: Enzyme stability is a critical factor in many biocatalytic applications. The inherent metastability of most native and engineered enzyme structures can hinder their effectiveness, making it essential to develop novel strategies to enhance the biocatalysts' stability for industrial uses (Garcia-Pardo et al., 2024).

Amyloids possess several characteristics that make them ideal scaffolds for developing novel nanozymes, such as robustness, affordability, and ease of customization. Additionally, the assembly of molecules into ordered fibrillar structures provides periodicity and a high morphological aspect ratio, bringing multiple functional groups into close proximity. This spatial arrangement amplifies the binding potency to other molecules of interest through avidity (Garcia-Pardo et al., 2024). Their structural characteristics, including modularity, high stability, and reusability, hold immense potential for applications in biotechnology, nanotechnology, and material science, including the development of novel biosensor devices, purification systems, drug delivery systems, energy converters, and nanozymes (**Figure 1**; Wei et al., 2017; Navarro et al., 2023; Peña-Díaz et al., 2024). Among these applications, their role as catalysts stands out due to their versatility in facilitating chemical reactions.

In the quest for the minimal catalytic entity: Although amyloid aggregates were initially identified for naturally occurring proteins, a reductionist approach has emerged to identify the minimal aminoacidic fragment capable of forming catalytically active assemblies. As mentioned above, in 2014, Rufo et al. identified a series of seven-residue peptides capable of forming catalytic assemblies. Using rational design, they produced amyloid structures with exposed reactive surfaces along the fibril axis, mimicking the active sites of natural enzymes. In a similar vein, Díaz-Caballero et al. (2018) designed four polar binary patterned peptides with the [Q/N/G/S]-Y-[Q/N/G/S] motif, frequently observed in naturally occurring prion-like sequences. More recently, the same group developed a related enzyme-mimetic nanomaterial that assembled in a pH-dependent manner, allowing enzymatic reactions to be modulated by altering the solubility of the peptides through pH changes, highlighting a tight relationship between amyloid structure and activity (Díaz-Caballero et al., 2021). The activity of synthetic amyloid catalysts often depended on the presence of metal ions acting as cofactors. For instance, this first generation of synthetic amyloid nanozymes was functionalized with metal cations like Zn^{2+} or Cu^{2+} to perform the respective enzymatic reactions. To achieve metal coordination, the active site of these enzymes often encompassed His residues, responsible for metal ion binding (Rufo et al., 2014; Díaz-Caballero et al., 2018, 2021; Garcia-Pardo et al., 2024).

Taking this approach even further, Makam et al. (2019) identified the shortest entities capable of forming catalytic amyloids. They developed a minimal enzyme involving a single Phe amino acid that self-assembles into amyloid fibrils with prominent catalytic activities in the presence of Zn^{2+}.

Developing synthetic hydrolases: Over the past decade, several other studies have investigated the use of short amyloidogenic peptides as artificial metalloenzymes, primarily for hydrolytic reactions (**Figure 2**). A remarkable example is the recent development of a self-assembled catalyst exhibiting both esterase and carbonic anhydrase activities (Navarro et al., 2023). Unlike other reported catalytic amyloids, this system utilizes Tyr residues to coordinate with divalent metal cofactors such as Cu^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}. Notably, this catalytic system binds metal cations in its mature fibrillar state while assembling independently of these ions. This duality allows the material to function both as a nanoenzyme and as a metal scavenger (Navarro et al., 2023).

Other amyloids with catalytic activity: In an alternative approach, Su et al. (2024) have recently described a strategy to develop amyloid catalysts for clinical purposes. In their study, they created a catalytic amyloid hydrogel derived from the milk protein β-lactoglobulin. Upon coordination with iron ions, this nanozyme efficiently catalyzed alcohol oxidation, outperforming current natural enzyme complexes and avoiding the accumulation of toxic metabolites. When orally administered to mice, this catalyst demonstrated alcohol detoxification and a protective effect on the liver, laying the groundwork for the design of artificial enzymes for therapeutic applications and clinical translation.

Besides the above-mentioned, tailor-made, *de novo*-designed peptides, different natural amyloidogenic protein sequences can also harbor inherent catalytic activities. Intriguingly, pathological amyloid fibrils associated with neurodegenerative diseases, such as α-synuclein, amyloid-β, or glucagon polypeptides, have been shown to possess esterase and dephosphorylase activities (Horvath and Wittung-Stafshede, 2023). As a general trend, the activities of these pathogenic amyloids were comparable to those of rationally designed catalytic amyloids.

Collectively, these examples underscore the potential of amyloid assemblies in developing novel catalytic structures, with a wide array of applications in biotechnology and material sciences.

Conclusions and future directions: Amyloids have emerged as an attractive alternative for the design of novel biocompatible materials and catalysts. Over the past decade, the discovery that short-peptide sequences can self-assemble into functional amyloid structures with remarkable stability and catalytic activity has significantly advanced this field. Nevertheless, these naïve materials often display weak and unspecific catalytic activities, which should be further improved by targeted engineering, a task in which

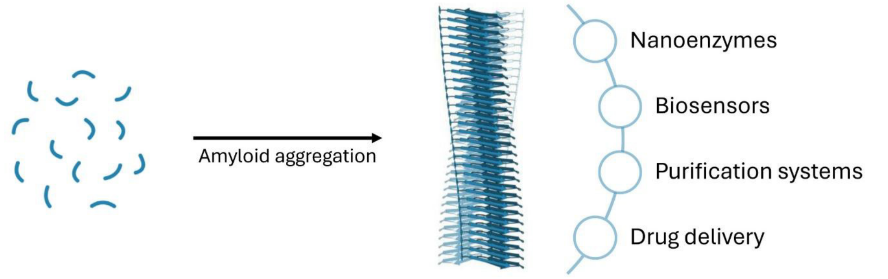

Figure 1 | Schematic representation of amyloids as functional scaffolds and their current biotechnological applications.
Scheme of an amyloid fibril self-assembly and examples of different applications as catalysts (nanoenzymes), biosensors, purification, and drug delivery systems. Created with BioRender.com.

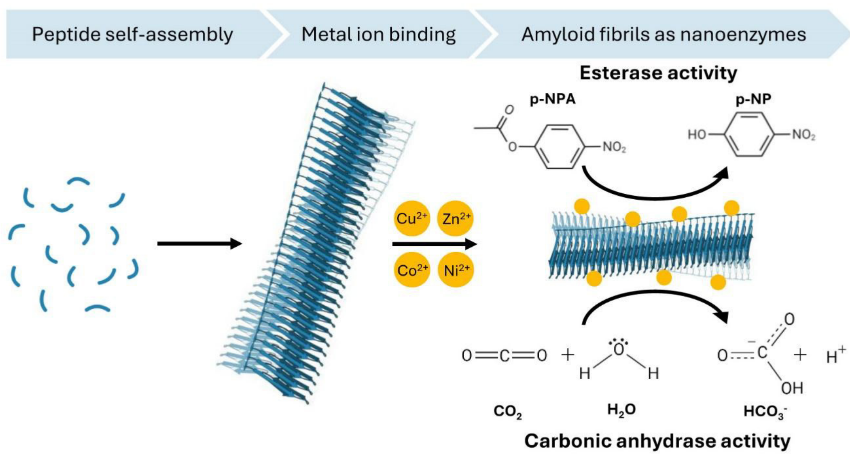

Figure 2 | Schematic representation of the self-assembly, functionalization, and enzymatic activity of artificial nanoenzymes.
Prion-inspired peptides can self-assemble into amyloid fibrils that, in coordination with divalent metal ions (Cu^{2+}, Zn^{2+}, Co^{2+}, or Ni^{2+}), display hydrolase activities, such as esterase or carbonic anhydrase. p-NP: p-Nitrophenol; p-NPA: p-nitrophenyl acetate. Created with BioRender.com.

generative artificial intelligence models such as AlphaFold 3 would likely play a central role.

To date, most reported natural and engineered catalytic amyloids exhibit hydrolytic activities, suggesting an inherent function arising from the intrinsic properties of the amyloid architecture. This structural disposition allows for the simple and efficient coordination of a variety of metal cofactors at their surfaces, with metal-dependent esterase being the most extensively exploited functionality in catalytic amyloids.

In this scenario, the rational design of catalytic amyloids with cofactor-independent activities presents a promising avenue to diversify the repertoire of catalytically active biomaterials. Despite significant advances in the field, obtaining atomistic structural and mechanistic insights into these systems remains a challenge. Such knowledge is essential for designing more complex and efficient amyloids that can rival naturally evolved globular enzymes. In this regard, recent advancements in cryo-electron microscopy have provided high-resolution images that reveal intricate details of the amyloid fibril structure and the catalytic core of amyloids with esterase activity (Heerde et al., 2023). This technique holds great promise for deepening our understanding of structure-function relationships in catalytic amyloids, paving the way for the development of more sophisticated and efficient amyloid-based biocatalysts.

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