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Conformational evolution of polymorphic amyloid assemblies

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The morphological diversity of amyloid assemblies has complicated the development of disease therapies and the design of novel biomaterials for decades. Here we review the conformational evolution of amyloids from the initial liquid–liquid phase separation into the oligomeric particle phase to the nucleation of the more ordered assembly phases. With mounting evidence that the assemblies emerging from the oligomeric phases may not be stable in solution and undergo further structural transitions, we propose the concept of conformational evolution, where mutations may occur at the ends or on the surface of the pre-existing fibers and different morphologies are under selection throughout the assembly process.

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Introduction

The polymorphic nature of amyloid continues to complicate the development of therapeutics for at least 50 different diseases [1]. Despite the common cross-β architectures of these assemblies [2], which suggests similar assembly mechanisms, these proteins access different morphologies or phases [3] that cannot be precisely distinguished with current disease biomarkers [4]. Also, the etiologically relevant structures remain to be defined, and there is little correlation between disease progression and amyloid deposition [5]. These unsolved problems have limited not only the development of disease therapies, but also the development of these unique biomolecular assemblies for other uses [6].

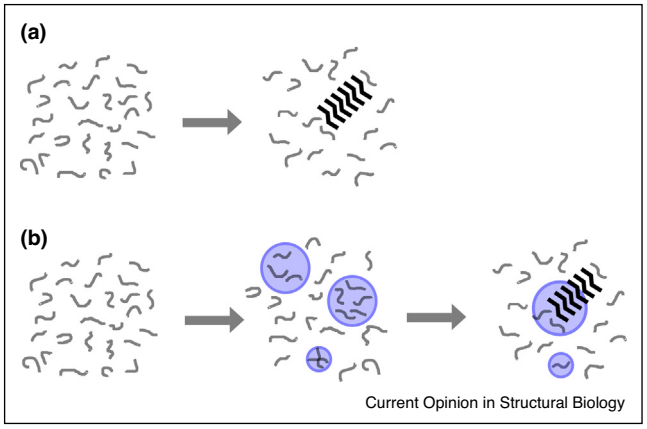
An increased understanding of various assembly intermediates has been obtained through advances in biophysical methods [7]. The intermediate species that are different from the final assemblies were initially observed with infrared spectroscopy (IR), circular dichroism (CD) and epitope mapping [8–10], complemented by imaging techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM) [11]. More recently, atomic resolution molecular structures of amyloids were obtained with solid-state NMR [11], cryo-EM [12] and X-ray analyses of microcrystals [13]. These methods not only reveal the polymorphic nature and the complex reaction pathways of amyloid assemblies [14•,15], but also clarify the dynamic structural transitions that occur during the assembly process. This idea of 'conformational evolution' may well underpin the robust nature of neurodegenerative disease progression, open new avenues for therapeutic intervention, and provide approaches for the construction of novel nanomaterials. Here we deconstruct the process by summarizing recent experimental interrogation of the assembly process and reveal the mechanisms for conformational evolution.

Assembly pathway

Peptide condensation and assembly generally follow a nucleation/propagation assembly pathway [16•,17]. Similar to crystallization, an initial nucleus is required to template assembly; classical nucleation theory [18] predicts that peptides nucleate into stable and ordered assemblies directly in solution (Figure 1a) [19]. However, strong aqueous desolvation energy barriers may slow down or prevent this direct single-step transition [20]. Rather, metastable intermediary phases appear to arise first via liquid–liquid phase separation, creating a different environment for the formation of the more ordered assemblies. By Ostwald's rule of stages [15], this initial phase separation yields the oligomeric peptide particles [21,22], while these metastable particles further initiate the nucleation of the paracrystalline assemblies [16•,20,23,24]. In this non-classical or two-step nucleation mechanism (Figure 1b), two distinct steps are required to access the final assembled structure.

Once a stable nucleus forms, it propagates through template-directed assembly with its growing ends recruiting free peptides, and thus the total number of ends strongly impacts assembly kinetics. Under conditions where the assemblies are vulnerable to shear forces from the environment, fiber fragmentation can significantly accelerate the assembly rate by making more ends [25]. Also, the assemblies may nucleate on other environmental

Figure 1



One-step and two-step nucleation pathways. **(a)** Classical one-step nucleation where stable nuclei are formed directly in solution, and **(b)** non-classical two-step nucleation where a metastable oligomeric peptide particle creates an environment for the formation of stable nuclei.

surfaces, and such surface nucleation events are expected to show distinct kinetics from fiber fragmentation [19]. Indeed, the environment in the complex cellular matrix is expected to play a critical role in each step of the assembly process, complicating any potential control over final assembled structure and potentially increasing the robust plasticity of any pathogenic amyloid structures.

Oligomeric intermediates

The initial liquid–liquid phase separation requires a critical concentration [7,9], and the oligomers have been distinguished from the mature assemblies by antibody binding, IR signatures, and cytotoxicity [9,11,26–29]. Ahmed *et al.* [11] reported that the Aβ(1–42) peptide forms intermediary pentameric assemblies with a three-turn structure, while the final assembly possesses a two-fold structure with an extended C-terminus. This Aβ(1–42) peptide has also been reported to form oligomeric intermediates with both parallel and anti-parallel β-hairpin structures, but the anti-parallel assemblies later diminish and the final mature fibers contain only parallel β-sheets [27]. For Aβ(1–40), its intermediary oligomers and protofibrils are stabilized by an intramolecular interaction between Glu22 and Ile31 [30], and a significant structural re-arrangement is required to transform these intermediates to mature fibers. This re-arrangement, and the following fiber maturation, may be prevented by trapping the oligomeric intermediates with disulfide bridges through incorporation of Cys residues [10,27,31]. On the other hand, the fibrillization propensity of Aβ(1–40) is increased when a lactam bridge is introduced between Asp23 and Lys28 residues to replace the proposed salt bridge [32], which stabilizes the oligomeric intermediates for the later fiber transformation.

As shown in Table 1, similar transitions occur with simpler peptides [14•,33]. The nucleating core of amyloid beta sequence, Aβ(16–22), and its congeners have been shown to assemble through intermediary structures before transitioning into their final phases. KVKVLGDVIEV forms out-of-register hexamers with β-barrel structures, which later mature into in-register fibers [34]. Hauser and coworkers found that Ac-ID_3 and Ac-LD_6 form oligomeric α-helical structures that then transition into the β-type structures [35]. These suggest that many peptides have access to diverse areas of the

Table 1

Summary of structural transitions during the assembly pathway

Sequence	Intermediate	Final Structure	Characterization method	Ref.
Aβ(1–42)	Pentamer with a three-turn structure	Parallel β-sheet with one turn	NMR, AFM, and SDS-PAGE	[36]
Aβ(1–42)	Switch between twisted and straight fibers		AFM	[37••]
Aβ(1–42)	Mixed anti-parallel and parallel	Parallel	NMR, ELISA, and AFM	[27]
Aβ(1–40)	E22 and I31 are in close proximity in protofibrils	E22-I31 contacts are absent in mature fibrils	ss-NMR	[30]
Aβ(16–22)E22Q	Anti-parallel out-of-register	Parallel	IE-IR, TEM, and ss-NMR	[33]
Aβ(16–22)	Anti-parallel out-of-register	Anti-parallel in-register	IE-IR and TEM	[38]
Aβ(16–22)	Mixed regions with different fluorescent lifetimes		Fluorescent lifetime imaging	[39]
hPrP(90–231)	Large oligomers with SDS only or small oligomers with SDS + NaCl	Fibrils only mature from the small oligomers	FRET, AFM, Raman spectroscopy, CD, DLS, cell viability	[40]
KVKVLGDVIEV	Hexamer with out-of-register β-sheet structure	Fibrils with in-register β-sheet structure	XRD, native nanoelectrospray mass spectrometry, and TEM	[34]
Ac-LD_6, Ac-ID_3	α-Helical oligomers	Fibers with β-sheet structures.	CD, SEM, and XRD	[35]