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Emerging biophysical origins and pathogenic implications of amyloid oligomers

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The amyloid hypothesis has been a leading narrative concerning the pathophysiological foundation of Alzheimer's and Parkinson's disease. At the two ends of the hypothesis lie the functional protein monomers and the pathology-defining amyloid fibrils, while the early stages of protein aggregation are populated by polymorphic, transient and neurotoxic oligomers. As the structure and activity of oligomers are intertwined, here we show oligomers arising from liquid-liquid phase separation and β-barrel formation, their routes to neurodegeneration, and their role in cerebrovascular perturbation. Together, this *Perspective* converges on the multifaceted oligomer-axis central to the pathological origin and, hence, the treatment of amyloid diseases.

The term "oligomer" refers to the association of a few monomers, per its Greek origin. In polymer chemistry, oligomers are linear, cyclic, branched, or globular molecules composed of repeating monomeric units, strung together by covalent forces or hydrogen bonds. In polymer physics, oligomers are beads on a string possessing a finite persistence length, interacting intra- and inter-molecularly via electrostatics in good or poor solvents. In biology, DNA and RNA oligonucleotides are opposing strands held together by hydrogen bonds and stabilized by π-stacking and polyelectrolytes. In all these cases, the oligomers are structurally defined, synthetically or naturally constructed to serve a specific engineering, physical, or biological purpose. But what exactly are the structure and purpose of the oligomers of amyloid proteins and peptides ("amyloid proteins" for brevity)?

Within the scope of amyloid science, oligomers denote a few to tens of monomeric amyloid proteins assembled together via non-specific forces and hydrogen bonding, assuming globular, curvilinear, branched, or annular morphologies on the nanoscale[1,2]. Such oligomers are heterogeneous and transient in structure, and their precise roles remain vague in cell biology and medicine. Small protofibrils, formed en route to amyloid fibrils, are sometimes classified under the same envelope as oligomers[3], possibly due to their shared features in small size and entailed toxicity.

How oligomers arise from monomers through physical forces under quasi-equilibria remains one of the most important, fascinating and yet controversial topics in amyloid protein science. The amyloid hypothesis was proposed by Hardy and Higgins[4] in 1992, depicting the kinetic processes of primary nucleation, elongation, and saturation of converting monomeric amyloid proteins into amyloid fibrils. This influential paradigm has since gone through major modifications to accommodate secondary nucleation[5], liquid-liquid phase separation (LLPS)[6], as well as cross seeding[7] each of which contributing to amyloid aggregation. Structurally, an amyloid protein, with the notable exception of tau, typically consists of three distinct regions: an N-terminus, a primary (and, sometimes, a secondary) amyloidogenic fragment, and a C-terminus. Environmental factors such as the cell membrane, metal ions, chaperone proteins, temperature and solvent pH also contribute to the aggregation dynamics of amyloid proteins[8,9]. The N-terminus of amyloid-beta (Aβ), a peptide associated with the pathology of Alzheimer's disease (AD), can initiate association of the peptide with the cell membrane to trigger structural transitions

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towards β-sheet rich oligomers and cross-β fibrils[8]. The C-terminus of $A\beta_{42}$, on the other hand, enhances the amyloidogenic potential of the peptide[10]. As the concentration of amyloid proteins rises above a threshold, prompted by pathophysiological conditions or environmental triggers, amyloid proteins sample through a host of conformations along the free-energy landscape, transforming from monomeric to oligomeric, protofibrillar and fibrillar states driven by thermodynamics, as observed time and again in vitro and in vivo. In this article, we focus on the LLPS process and the monomer to β-barrel evolution in oligomer formation (Fig. 1), as primary and secondary nucleation in amyloidosis have been extensively reviewed elsewhere[1,11,12].

Fundamentally, the biological and pathological footprints of proteins are inseparable from their structural characteristics. While the function of monomeric amyloid proteins is often ambiguous, Aβ contributes to the modulation of synaptic function[13] while human islet amyloid polypeptide (hIAPP, associated with type 2 diabetes or T2D) sustains glucose homeostasis[14]. In comparison with the intrinsically disordered monomers, the oligomers and small protofibrils of Aβ are structurally more diverse and biochemically unstable, actively engaging in neuronal damage through the induction of oxidative stress and inflammation[15–17]. Indeed, although our knowledge of the structure-function-toxicity trilogy of oligomers remains deficient and even disjointed, the pivotal roles of oligomers and protofibrils in neurodegeneration have recently been vindicated by the modest success of monoclonal AD drug lecanemab[18], which targets these conformations in early-stage AD patients. Accordingly, the oligomer-neurodegeneration axis warrants a discussion in this article (Fig. 1).

Cerebrovascular damage arising from amyloid aggregation has been an ongoing topic in research on AD and dementia. The working paradigm is that the oligomers and, to a lesser extent, the amyloid fibrils of Aβ, can incite reactive oxygen species (ROS) and trigger inflammation to compromise integrity of the vasculature, including the blood-brain barrier (BBB)[19–22]. These findings, while important to our elucidation of the curious correlation between AD and cerebral amyloid angiopathy (CAA)[19], have focused on intermediate to long exposures to oligomers and fibrils and hence are entangled with the toxicity biomarkers of amyloidosis. This is in contrast to our recent finding of amyloid protein-induced endothelial leakiness (APEL)[22], a paracellular phenomenon which occurs on the timescale of minutes independently of cellular ROS production, apoptosis, or autophagy. While the finding of APEL (Fig. 1) has opened a window to our elucidation of cerebrovascular damage and cross seeding in connection with AD and Parkinson's disease (PD) pathogenesis, it has further exposed our limited understanding of oligomers, a nexus for decoding amyloid diseases and their prevention.

In this work, we examine the early aggregation of amyloid proteins through LLPS and β-barrel formation, exploring their connections to neurodegeneration and their biophysical-biochemical interactions with adherens junctions in triggering cerebrovascular

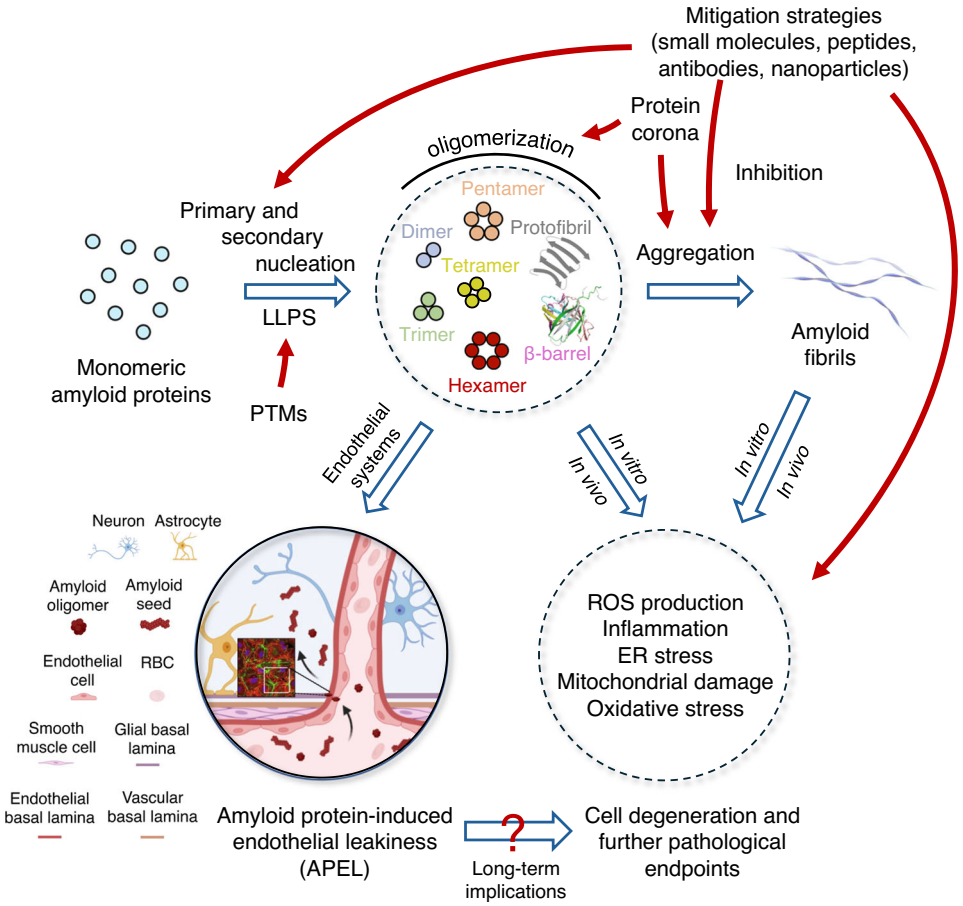

Fig. 1 | This *Perspective* reflects on the multifaceted and often confounding oligomer-axis of amyloid diseases. Specifically, we highlight the biophysical processes of liquid-liquid phase separation (LLPS) and β-barrel formation in amyloid protein oligomerization and illustrate the biological-pathological implications of oligomers via their entailed neurodegeneration and endothelial leakiness. We also discuss the roles of post-translational modifications (PTMs) and the protein corona on oligomer stability and toxicity. In addition, we present the state-of-the-art mitigation strategies against amyloid diseases. RBC red blood cell, ROS reactive oxygen species. The β-barrel structure in the illustration is reproduced with permission from ref. 46. Copyright 2021 Elsevier. The monolayer is reproduced with permission from ref. 22. Copyright 2024 Springer Nature. Created in BioRender (https://BioRender.com/r19r265).

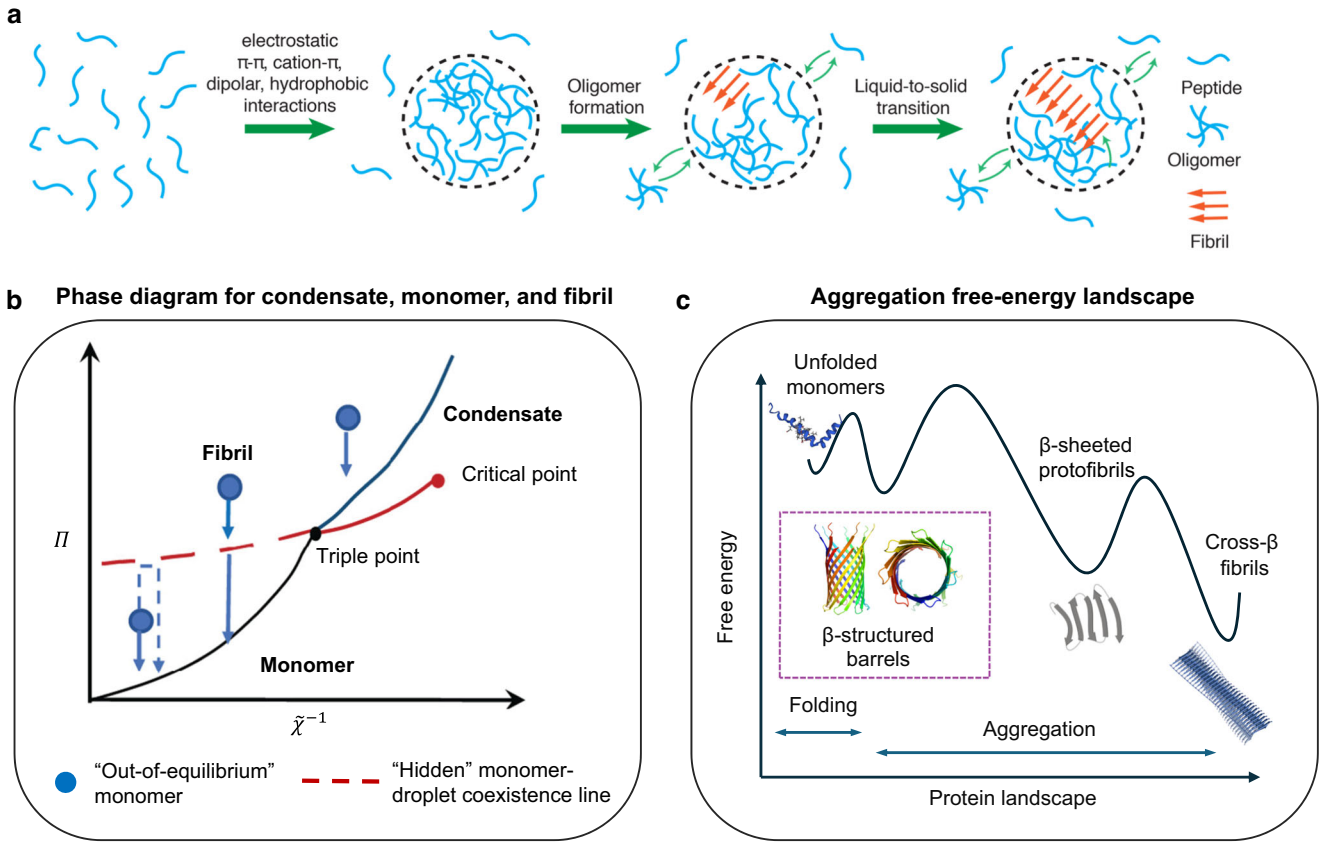

Fig. 2 | Amyloid oligomers in LLPS and formation of β-barrel pore intermediates. a Schematic of amyloid nucleation through LLPS. **b** Phase diagram of protein condensate, monomer, and fibril states. **c** Aggregation free-energy landscape illustrating that β-barrel pore intermediates are local minimal energy basins, formed in early aggregation stages. β-barrel structure (PDB: 7O1Q), Aβ monomer (PBD: 1IYT). **b** is reproduced with permission from ref. 28. Copyright 2021, American Chemical Society. **c** Created in BioRender (https://BioRender.com/d55o699).

permeability. This Perspective highlights the multifaceted and yet converging oligomer-axis underlying the pathophysiology, diagnosis, and treatment of amyloid diseases.

Protein aggregation, neurodegeneration, and endothelial leakiness

Liquid-liquid phase separation

Amyloid protein aggregation through LLPS. Mounting evidence suggests that a wide range of diseases-associated proteins, including hIAPP, Aβ, tau, α-synuclein (αS), transactive response DNA binding protein 43 (TDP-43) and heterogeneous nuclear ribonucleoprotein A1 and A2 (hnRNPA1/2), undergo LLPS by spontaneously partitioning a homogenous solution into a high-density phase (liquid-like membraneless condensates) and a co-existing low-density phase[6] (Fig. 2a). These protein-rich condensates, usually referred to as "droplets", provide temporal and spatial organizations of amyloid proteins. LLPS is essential to normal cellular function and the pathological processes underlining multiple neurodegenerative diseases[23].

The formation of condensates is mediated by multivalent, low-affinity interactions involving repetitive modular domains and intrinsically disordered regions containing weakly adhesive motifs frequently found in proteins linked with neurodegenerative diseases. These interactions, which drive LLPS, include electrostatic, π-π, cation-π, dipolar or hydrophobic interactions between aromatic, polar and charged residues. Together, they create dynamic and reversible microenvironments critical for physiological functions and pathological aggregation. Moreover, the LLPS of amyloid proteins can be mediated by familial mutations, exposure to metal ions, PTMs, and other disease-promoting factors[24]. In brief, interactions driving LLPS are highly protein-specific and environmentally dependent that deserve further investigation.

Oligomer formation under the lens of LLPS. Condensates formed by LLPS exert a significant influence on the kinetics of amyloid aggregation, evidenced by the disruption of LLPS retarding amyloid aggregation of the TDP-43 low complexity domain[25] and the LLPS of Aβ oligomers conversely promoting Aβ fibrillization[26]. Theoretically, the oligomerization and aggregation of amyloid proteins can be described within the framework of LLPS. According to the Flory-Huggins theory, the LLPS of amyloid proteins features binodal and spinodal curves, which identify the characteristic binodal (B) and spinodal (S) points at low (L) and high (H) concentrations with $\phi_{BL} < \phi_{SL} < \phi_{SH} < \phi_{BH}$. Specifically, when the peptide concentration is within $\phi_{BL} < \phi < \phi_{SL}$, the peptides aggregate into metastable oligomers that frequently exchange with peptide monomers in solution, or nanodroplets of the high-density liquid phase (HDLP)[27]. As the peptide concentration increases ($\phi_{SL} < \phi$), the condensates enlarge and stabilize by partitioning more peptides into the HDLP. Importantly, peptides follow the classic nucleation-elongation pathway at concentrations below ϕ_{SL}. In contrast, peptides above ϕ_{SL} undergo LLPS first by forming non-fibrillar HDLP, with subsequent nucleation and elongation of fibril seeds within the condensates. Here, oligomerization and fibrillization are governed by high local peptide concentrations within the condensates, indicating that the amyloid aggregation time will saturate to a constant as peptide concentration increases[27].

From a broader perspective, amyloid aggregation and LLPS can be interpreted within the unified theory of phase transition by analogizing to the gas-liquid phase transition system[28]. Based on the phase

diagrams, amyloid aggregation can be viewed as an evolution from the initial "out-of-equilibrium" state of a homogeneous solution of monomers to the final equilibrium state of amyloid fibrils coexisting with monomers and/or droplets (Fig. 2b). During equilibration, the monomer-droplet, droplet-fibril, and monomer-fibril phase transitions are involved in the kinetic nucleation pathway depending on the monomeric state's initial position within the phase diagram. Meanwhile, the crowded internal structure, viscosity, heterogeneity, and time-varying fluidity of condensates create a distinct environment conducive to the formation of oligomers, which necessitates the development of models accounting for amyloid oligomerization within these condensates.

Structure and evolution of oligomers entailed by LLPS. To uncover the structure and evolution of oligomers in LLPS is especially difficult considering the dynamic and transient features of LLPS. Oligomers at the early stages can act as a nucleus for the growth and coalescence of condensates, resulting in a single large droplet or multiple small-sized condensates that are stable over time[29]. The final fate of the oligomers is determined by competition between the time when proteins diffuse into contact and the time for sticker regions of the peptides to form and break physical bonds[29]. When diffusion is orders of magnitude faster than bond formation, inter-sticker bonds are formed mainly within small condensates. Exhaustion of available valences within smaller condensates prevents growth of the condensates, resulting in the prevalence of stable small- and medium-sized condensates[29]. Furthermore, depending on the ratio of hydrophobic to hydrophilic sequence components, condensates can adopt different morphologies including micelles, tubes, lamellae, and network structures[30], further modulating the kinetics of amyloid aggregation.

The internal structures of condensates are closely related to peptide percolation. When the peptide concentration of the condensates is above the percolation threshold concentration, LLPS spontaneously leads to percolation in condensates, forming porous and network-spanning structures[31]. Inter-peptide contacts within the network are heterogeneous, characterized by low peptide connectivity and predominantly weak residue interactions. The high peptide connectivity and strong residue contact states, which can be regarded as oligomers formed inside the condensates, are rare but crucial for fibrillization and secondary structure conversion. Together, oligomers formed under LLPS are dynamic, entailing rich heterogeneous conformations and internal topologies.

Pathologies of oligomers and therapeutic treatments targeting LLPS. Disruption of protein homeostasis in maintaining the balance between native, amyloid, and condensate states underlies the pathology of numerous neurodegenerative diseases. Common failures include disturbances in LLPS and liquid-to-solid transitions, often driven by disease-associated mutations or PTMs to result in toxic protein aggregation and cellular dysfunction. For instance, aberrant phase-separated ribonucleoprotein condensates can impair neuronal function through several mechanisms: mislocalization to the cytoplasm, loss of liquid-like properties that disrupt mRNA transport, sequestration of mRNAs and RNA-binding proteins to dysregulated translation, and eventual transition to pathological fibrillar aggregates[32].

Pathologies arising from the structural evolution of amyloid proteins are closely linked to the oligomer species. Toxic oligomers are observed to accumulate within the droplets during the liquid-to-solid transition of αS aggregation[33]. Similarly, condensates of tau also accumulate off-pathway toxic oligomers during the liquid-to-solid transition, which do not irreversibly convert to amyloid fibrils[34]. Oligomers formed within liquid-like condensates are expected to diffuse and interact dynamically with their surrounding environment, facilitating their release and enabling membrane interactions that contribute to their toxicity.

The pathologies of neurodegenerative diseases can be triggered by the phase separation of coexisting biomolecules, which elevates protein concentration and promotes oligomer formation and aggregation. For example, the prion protein associated with Creutzfeldt-Jakob disease formed dynamic heterotypic liquid droplets with αS, a protein linked to PD via LLPS[35]. Maturation of droplets into heterotypic amyloid fibrils could be responsible for overlapping neuropathological features observed in neurodegenerative diseases. In this context, αS was found to form clusters on the surface of TDP-43 low complexity domain-RNA droplets, highlighting a potential molecular mechanism for the colocalized deposits of TDP-43 and αS in frontotemporal lobar degeneration, Lewy body disease, and multiple system atrophy[36].

The pathological links between aberrant LLPS and cellular and molecular dysfunctions in neurodegenerative diseases inspire therapeutic approaches targeting biomolecular condensates. Several strategies have been proposed, using small molecules, peptides, molecular chaperones, nanomaterials, and protein-aggregate degraders[37]. These approaches aim to modulate the biophysical properties, macromolecular composition, dynamics, and/or free-energy landscape of condensate assembly and disassembly.

β-barrels, a distinct subclass of oligomers

β-barrel intermediates as a toxic oligomer species. Amyloid oligomers represent a mixture of small aggregation intermediates with continuous distributions in size and secondary structure, not all of which are cytotoxic. Based on the structure-function principle of molecular biology[38], toxic oligomers are believed to have well-defined three-dimensional structures for carrying out their pathological activities. The implication of a common amyloid toxicity mechanism, given the shared symptomology of various amyloid diseases, also suggests that toxic oligomers of different amyloid proteins may share similar structural features. In 2002, Lashuel et al. reported their observation of pore-like "protofibrils" of αS mutants A30P and A53T and of Aβ mutant Aβ-arctic[39]. These distinct structures, rich in β-sheets, were fractionated by gel-filtration chromatography and analyzed by electron microscopy, displaying an outer diameter of 7–12 nm and an inner diameter of 1.5–2.5 nm and comprising approximately 20–60 monomers each. These annulars notably resembled the cytolytic β-barrel pore-forming toxins from *Clostridium perfringens* and were connected with the poration activity of amyloid proteins observed in in vitro membrane systems[39]. Atomic force microscopy imaging of Aβ, αS, serum amyloid A, and hIAPP revealed that various amyloid proteins formed pore-like structures, disrupting the membrane to cause leakage[40]. In concordance with this, numerous studies have demonstrated that all amyloid proteins can generate common pore oligomers that cause lipid membrane leakage[41–43]. These β-barrel oligomers—possessing a well-defined 3D structure, the capability for membrane incorporation, and an alignment with the "amyloid-pore" hypothesis of toxicity[39,44]—have been proposed as toxic oligomer species in amyloid aggregation.

β-barrels as common aggregation intermediates. The structure, assembly dynamics (Fig. 2c), and toxicity of β-barrel intermediates have been investigated for many amyloid proteins. Notably, using all-atom discrete molecular dynamics (DMD) simulations, the fibrillization processes of amyloid protein fragments with contrasting cytotoxicities have been investigated for $hIAPP_{19\text{-}29}$, $hIAPP_{19\text{-}29}$ (S20G), $hIAPP_{22\text{-}28}$, $hIAPP_{8\text{-}20}$, $Aβ_{16–22}$, NACore (an 11-residue fragment of αS), and $SOD_{128–38}$ versus non-toxic peptides including $hIAPP_{15\text{-}25}$, $hIAPP_{15\text{-}25}$ (S20G), $SOD_{128–38}$ (G33W), and $SOD_{128\text{-}38}$ (G33V)[45]. *Only* the toxic amyloid proteins formed β-barrel intermediates en route to fibrillization.

In addition, formation of β-barrel intermediates during the aggregation of full-length toxic amyloid proteins—such as hIAPP, Aβ, and medin—is also evidenced[46,47]. A correlation between

experimentally observed cytotoxicity and formation of β-barrel intermediates in silico is supported by studies employing both fragmental and full-length peptides[46,48], pointing to β-barrels being a toxic oligomer species. Furthermore, rapid DMD simulations have revealed β-barrel oligomers formed by Aβ co-aggregating with hIAPP, αS, or medin, reinforcing the potential role of β-barrel structures as a toxic species in amyloidosis[47]. Given the presence of multiple amyloid disease-related peptides within the same pathological tissues or organs (e.g., hIAPP and medin in AD plaques, and Aβ in PD Lewy bodies) and the co-occurrence of multiple amyloid pathologies within the same individuals (e.g., AD with PD, T2D with AD), these findings entail profound implications for diagnosis and treatment.

Effects of LLPS on β-barrel oligomer formation. While LLPS is a phenomenon independent of the amyloidogenic sequence, it still plays a critical role in driving amyloid aggregation. For instance, both amyloidogenic hIAPP and non-amyloidogenic rIAPP exhibited LLPS in vitro, but localized high concentrations of hIAPP progressed to a solid fibril state while rIAPP did not[49]. The dynamic helical accumulation of hIAPP led to a conformational conversion toward β-sheet aggregates, accompanied by the formation of β-barrel intermediates[50]. In contrast, rIAPP only aggregated into dynamic helical oligomers that frequently dissociated; their conformational conversion into β-sheet structures was rare and reversible. These observations indicate that high local concentrations, as exhibited in LLPS, are key to the formation of β-barrel intermediates and cross-β fibrils. The inability of rIAPP to form β-barrel structures stems from its intrinsic sequence properties rather than the limitations of LLPS itself. While LLPS can create favorable conditions for nucleation, the transition to toxic aggregates ultimately depends on the amyloidogenic characteristics of the peptides.

Diagnostic and therapeutic values of β-barrel research. Future diagnostics and treatments for amyloid diseases may benefit from key insights into β-barrel oligomer research. These oligomers are believed to be more toxic than fibrils or plaques due to their ability to induce membrane poration, leading to mitochondrial dysfunction and calcium dysregulation. Targeting these toxic intermediates could help prevent early neuronal damage. Additionally, the development of antibodies that selectively target β-barrel oligomers without interfering with physiological protein functions may offer precise therapeutic strategies. Preventing β-barrel oligomers from acting as aggregation seeds could also mitigate their role in pathology propagation and neuroinflammation, potentially slowing disease progression. Furthermore, the identification of oligomer-specific biomarkers in cerebrospinal fluid (CSF) or blood could enable early diagnosis and timely intervention. Beyond AD, these insights may have broader implications, extending to other neurodegenerative diseases such as PD and Huntington's disease (HTT), thereby opening avenues for treating a range of proteinopathies.

Amyloid oligomers and neurodegeneration

With our prior discussions on the biophysical inner workings of oligomerization, this section makes the crucial connection between the chemistry, structure and toxicity properties of amyloid protein oligomers.

Extensive literature has shown that the accumulation of amyloid proteins, especially the formation of oligomers, is causative to ROS production, cerebral inflammation, disrupted synaptic transmission and plasticity, and dysregulated neuronal protein homeostasis[51–55]. Synaptic failure is an early event in the pathology of neurodegeneration, as observed in patients with mild cognitive impairment[51,54]. These findings supplement recent clinical evidence that Aβ oligomers within CSF are significantly elevated in individuals with mild cognitive impairment[56], based on the six-stage symptom classification of patients according to the National Institute on Aging and the Alzheimer's Association research framework[57]. Decreased CSF levels of Aβ oligomers in later-stage patients may have resulted from the oligomer-to-fibril/plaque transition or may be associated with the complex dynamics of Aβ metabolism in AD, including impaired brain-to-CSF Aβ clearance[56]. Hence, Aβ oligomers may be a key target for early therapeutic interventions prior to tau accumulation and neurofibrillary tangle formation in patients. Indeed, Aβ dimers extracted from the AD cortex have been reported to alter the phosphorylation state of epitopes within tau[58]. The specific stage during which tau undergoes PTMs is crucial, as current evidence indicates that the toxic state of tau can amplify upstream Aβ toxicity through a feedback loop[59].

Correlating the occurring events before and during oligomerization. To understand amyloid aggregation at the molecular level, it is crucial to explore the early events that drive oligomerization. The crowded environments of the cytoplasm and nucleus, with elevated protein concentrations and changes in ionic strength and pH, destabilize amyloidogenic proteins. These conditions lower the entropic cost of phase separation, making condensate formation favorable[24]. This increases the likelihood of intermolecular interactions, including transient contacts that lead to oligomer nucleation. Thermodynamically, LLPS and subsequent oligomerization of amyloidogenic proteins occur along a continuum, shaped by entropy-enthalpy balance[60]. Aggregation reduces dispersed particles in solution, triggering phase separation to restore entropy. Oligomerization forms metastable intermediates, such as β-barrel structures or spherical oligomers, overcoming the entropic penalty[61]. LLPS and oligomerization do coexist[26], with LLPS acting as an entropy-driven prelude to more enthalpically stabilized, entropically costly oligomeric states.

In membrane environments, β-barrel oligomers form pore-like structures that disrupt membrane integrity, causing ion leakage, calcium influx, and oxidative stress[62]. This mechanism underpins synaptic dysfunction and cell death, supporting the oligomer toxicity hypothesis[62]. However, despite compelling in vitro evidence, β-barrels have not been directly resolved from in vivo samples. The difficulty in purifying these structures limits understanding their distinct properties compared to spherical oligomers, and mixed oligomeric populations complicate the analysis of their toxicities. Aβ-derived diffusible ligands (ADDLs), found in higher concentrations in post-mortem AD brains, share similarities with β-barrel oligomers. ADDLs are small (5–45 nm), soluble, and diffusible oligomers present early in Aβ aggregation[63]. While β-barrel oligomers are structurally defined, their toxicity profiles differ from ADDLs. Specifically, ADDLs primarily disrupt synaptic signaling by interacting with NMDA and EphB2 receptors, impairing memory and learning. Purifying such oligomers is challenging, but studies show that annular αS species increase membrane permeability, elevate calcium levels, and induce cell death, while globular αS oligomers seed aggregation without these toxic effects[64]. Similarly, transthyretin studies suggest that annular species are transient intermediates[65]. The co-existence of annular and globular oligomers contributes to multiple toxicity mechanisms, from acute membrane disruption to chronic synaptic dysfunction.

Neurodegenerative impact and therapeutic relevance of oligomers. Understanding the neurodegenerative impact of amyloid oligomers requires a holistic perspective. While reductionist approaches have revealed their toxic roles—disrupting proteostasis, impairing mitochondrial function, and triggering programmed cell death—integrating these insights within cellular and intercellular networks is crucial for addressing the multifaceted contributions to amyloid diseases and their therapeutic mitigation.

Recent research has examined early events in amyloid-related proteotoxicity to address its progression and develop therapies[52]. Such key events include amyloid precursor protein (APP) cleavage by

β- and γ-secretases, extracellular Aβ accumulation, receptor-mediated internalization, and neuron-to-neuron transmission via endocytic vesicles[55]. Internalized Aβ oligomers accumulate in the endoplasmic reticulum (ER), causing misfolded protein buildup, ER stress, and activation of the unfolded protein response (UPR)[55]. The UPR, in turn, restores homeostasis by boosting molecular chaperones and protein degradation through pathways like the ubiquitin-proteasome system. While vital for neuronal function and memory, the proteasome can be inhibited by amyloid oligomers, which evade degradation and worsen proteostasis[66]. Targeted therapies, accordingly, may address proteasomal dysfunction, enhance UPR, and leverage mechanisms shared across amyloid oligomers for applications.

Mitochondria, critical for neuronal survival and bioenergetics, are particularly vulnerable to oligomer-induced damage, as evidenced by the co-localization of APP and Aβ with mitochondria[67]. Aβ oligomers depolarize mitochondrial membranes, trigger cytochrome *c* release, and activate apoptotic pathways. They disrupt calcium homeostasis by promoting excessive mitochondrial Ca^{2+} uptake, leading to ROS generation, ATP inhibition, and further cytochrome *c* release[68]. Additionally, oligomers also drive mitochondrial fragmentation through S-nitrosylation of dynamin-related protein 1 (Drp1), linked to elevated Drp1 levels in AD brains[69]. Fragmented mitochondria fail to maintain bioenergetic balance, exacerbating synaptic dysfunction and neurotoxicity. These effects extend to glial cells, where mitochondrial dysfunction intensifies inflammation and neuronal damage, accelerating neurodegenerative disease progression.

Necroptosis, a programmed cell death mechanism, has been implicated in neurodegenerative diseases like PD, amyotrophic lateral sclerosis (ALS) and HTT. Recent evidence highlights microglia-neuron interactions, showing microglia mediate Aβ oligomer-induced necroptosis[70]. Moreover, a critical link exists between necroptosis and tau pathology[71], with phosphorylated tau co-localizing with necroptosis markers in AD brains. Additionally, human neurons exhibit specific vulnerability, with Aβ plaques upregulating MEG3 noncoding RNA—a response absent in mice[72]. These findings underscore necroptosis's role in neurodegeneration and its interplay with synaptic dysfunction and mitochondrial damage, offering therapeutic opportunities.

Aβ oligomers are strongly implicated in AD pathology, prompting the development of transgenic mice with the E693Δ mutation, which promotes oligomerization over fibrillization[68]. Unlike wildtype APP mice, these models accumulate intracellular Aβ oligomers without forming extracellular plaques, causing tau phosphorylation, microglial activation, and neuronal loss[68]. In PD, L61 transgenic mice show severe motor impairments linked to increased αS oligomers[73]. These findings suggest oligomeric species drive disease progression, with Aβ oligomers potentially amplifying tau PTMs, independently of amyloid deposits. Similarly, elevated αS oligomers in Lewy pathology highlight their role in neurodegeneration, underscoring oligomers' pathological significance[74].

In reflection, oligomers play a pivotal role in early neurodegenerative progression, driving neuroinflammation, immune responses, cell signaling, and metabolic dysfunction. Their molecular heterogeneity makes these small entities promising but tricky therapeutic targets. Addressing protein aggregate interactomes and enhancing cellular degradation systems could offer strategies to mitigate disease progression and develop effective treatments.

Endothelial leakiness induced by oligomers

Impact of oligomeric Aβ on endothelial dysfunction. Toxic Aβ oligomers found within amyloid plaques are the primary agent of neurodegeneration, known to cause inflammation, oxidative stress, and impaired endothelial function—enhancing vasoconstriction and attenuating endothelium-dependent vasodilation[75,76]. These effects collectively reduce cerebral blood flow and cause overall cerebral vasculopathy[75,76]. In turn, cerebral hypoperfusion may accelerate amyloid accumulation, BBB dysfunction, and cognitive decline[77,78]. Studies have demonstrated the role of physiological Aβ levels in early AD in disrupting the endothelial function of perfused rat cerebral vessels and cultured endothelial cells, resulting in decreased nitrogen oxide production and reduced sensitivity to endothelium-dependent vasodilation and leading to vasoconstriction and ischemia in surrounding tissue[79]. Capillary constriction due to pericyte-mediated endothelial dysfunction was observed in biopsies from demented patients and rodents following the application of oligomeric Aβ[80,81]. Additionally, Aβ oligomers can impair mitochondrial function in endothelial cells, reducing ATP production and increasing ROS production[82].

BBB impairment by Aβ oligomers. Clinical studies have identified BBB damage as an early biomarker of cognitive impairment in AD patients[83]. AD-related changes in the BBB include degeneration of endothelial cells, reduced capillary length, serum leakage, altered expression and activity of BBB transporters, and disruption of tight junctions[84]. Unlike monomers or fibrils, $Aβ_{42}$ oligomers have been found to reduce levels of tight junction scaffold proteins—ZO-1, claudin-5, and occludin—and induce BBB leakage. This occurs in a time- and dose-dependent manner via upregulation of the receptor for advanced glycation end-products (RAGE) and metalloproteinases in vitro[85]. Aβ oligomers can also directly inhibit Wnt/β-catenin signaling in brain endothelial cells, further compromising BBB function[86]. A pronounced accumulation of $Aβ_{42}$ oligomers at the BBB has been demonstrated to disrupt tight junctions and cause leakage, as evidenced by a barrier integrity assay on a 3D microfluidic vasculature-on-a-chip model[87]. Treatment with $Aβ_{42}$ oligomers consistently reduced vessel formation and caused disruptions in both single brain endothelial cells and triple cultures designed to mimic the microvascular unit consisting of brain endothelial cells, astrocytes, and pericytes[88]. After stereotactic injection of oligomeric $Aβ_{42}$ in 2-month-old wild-type mice, elevated BBB permeability and local cortex hypoperfusion were notable[89]. Elevated inflammatory factors in pericytes following Aβ oligomer treatment aggravated the injury of tight junction proteins, suggesting that pericyte-associated BBB breakdown is critical to oligomer-induced vasculopathy.

Amyloid protein-induced endothelial leakiness (APEL). Recently, we have reported APEL, observed in intact monolayers of human microvascular endothelial cells (HMVECs) exposed to the oligomers and seeds of anionic amyloid proteins Aβ and αS[22]. In contrast, cationic hIAPP oligomers did not induce this paracellular effect, possibly due to their high affinity for cell membranes mediated via electrostatics. Notably, Aβ and αS oligomers, which were 10–70 nm in length and less than 20 nm in thickness, significantly increased endothelial permeability (Fig. 3a).

Mechanistically, APEL was initiated by single-molecular interactions between small amyloid protein aggregates and vascular endothelial cadherins (VE-cadherins), which collectively unzipped the paracellular space to create microscopic gaps over the exposure of tens of minutes[22]. This loss of paracellular connections alerted the signaling pathway of adherens junctions, and subsequently triggered remodeling of cytoskeletal proteins through the cadherin-actin mechanical "lever". Interestingly, significant downregulations of the tight junction proteins ZO-1, occludin, and claudin-5 were not observed upon their exposure to the protein aggregates, in contrast to previous findings under different experimental conditions[87]. In addition, the occurrence of APEL was found to be independent of the toxicity events of ROS production, apoptosis, and autophagy in vitro[22], a marked departure from literature reporting vascular damage induced by Aβ oligomers and fibrils on the timescales of hours to days[90].

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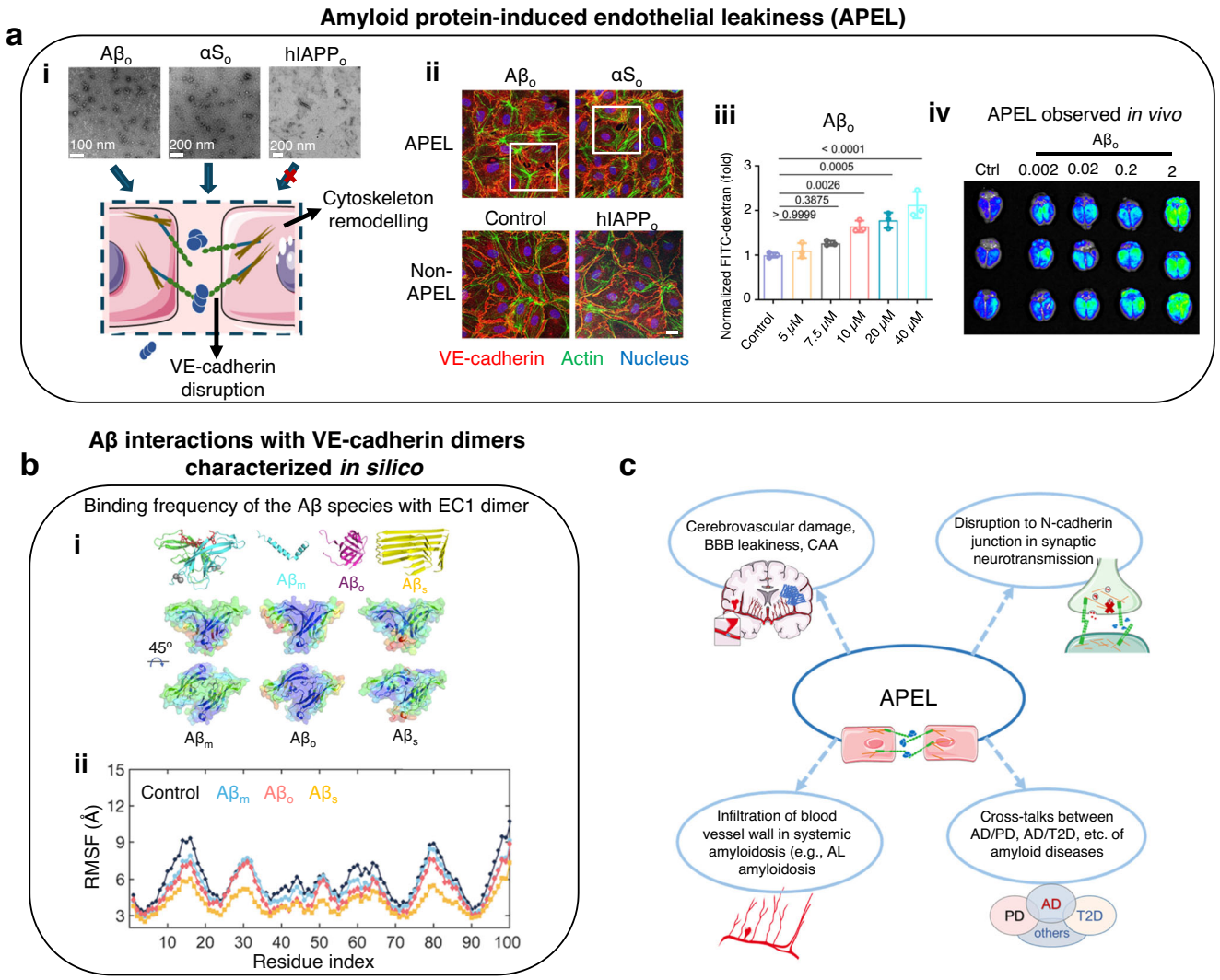

Fig. 3 | Phenomenon, molecular mechanism and implications/applications of amyloid protein-induced endothelial leakiness (APEL). a Pathogenic oligomers of Aβ, αS and hIAPP ($Aβ_o$, $αS_o$, $hIAPP_o$) and their differential capacities in eliciting APEL in vitro and in vivo. (i) TEM imaging of the oligomers. Illustration of APEL occurring in the intercellular space. (ii) Confocal fluorescence microscopy revealed endothelial leakiness with $Aβ_o$ and $αS_o$. No endothelial leakiness was observed in the presence of $hIAPP_o$ and control. Red: VE-cadherin, green: actin, blue: nucleus. Scale bars: 20 μm. (iii) A transwell assay revealed occurrence of endothelial leakiness after 30 min incubation with $Aβ_o$. (iv) In vivo injection of $Aβ_o$ into Swiss mice gave rise to an increased leakiness across the BBB after 24 h. **b** DMD simulations of the binding between the Aβ species and VE-cadherin dimers and steered DMD simulations of the cadherin-Aβ complexes. (i) Binding frequency of the Aβ species ($Aβ_m$: Aβ monomer; $Aβ_s$: Aβ seed) with the cadherin extracellular domain 1 (EC1) dimer. Blue and red on the EC1 dimer surface indicate low to high binding frequencies. (ii) Root-mean-square fluctuations (RMSFs) of the EC1 cadherin dimer with and without the Aβ species. **c** Potential implications/applications of APEL in neurodegenerative disorders, systemic amyloidosis and metabolic diseases. BBB blood-brain barrier, CAA cerebral amyloid angiopathy, AL amyloid light chain. **a**, **b** are reproduced with permission from ref. 22. Copyright 2024, Springer Nature. Some art elements in **a** and **c** are from Servier Medical Art, licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

In vivo, consistently, cerebrovascular permeability was increased in the mouse brain treated with Aβ oligomers, accompanied by the presence of the Aβ structures within the brain (Fig. 3a). VE-cadherin signaling was implicated, as PP1 treatment reduced endothelial leakiness indicating that APEL could be reversed in vivo[22]. On the molecular level, Aβ oligomers perturbed the outermost EC1/EC2 domains of the VE-cadherin dimer and increased the likelihood of early VE-cadherin dimer dissociation under low forces, reducing VE-cadherin stability to ultimately facilitate endothelial gap formation (Fig. 3b).

Implications and therapeutic relevance of APEL. The in vitro, in vivo and in silico findings collectively revealed the rapid and extracellular nature of APEL mediated by VE-cadherin signaling and actin remodeling. The phenomenon and mechanism of APEL are distinct from existing AD pathology, underscoring charge (anionic and/or neutral), size (nanoscale, to match the <100 nm paracellular gaps of adherens junctions), and perhaps rigidity as prerequisites for mediating the paracellular transport and permeability by the oligomers and seeds of amyloid proteins[22]. As research on APEL is still in the early stage, these observations do not preclude the possibility of long-term pathogenic implications. Furthermore, our findings point to a range of pathogenic implications as well as diagnostic and therapeutic applications of APEL concerning cerebrovascular damage, CAA, amyloid protein cross-seeding, vascular wall infiltration in systemic amyloidosis, and disruption to neurotransmission in AD/PD resulting from the paracellular transport of oligomers and protofibrils (Fig. 3c).

Outlook

For decades, research on amyloid aggregation has focused on its role in neuroinflammation, neurodegeneration, and synaptic dysfunction.

Table 1 | Clinically trialed immunotherapies and peptide vaccines against AD

Immunotherapy	Label	Characteristics	Targeting species					Clinical stage
			Monomers	Oligomers	Protofibrils	Fibrils	Plaques	
Passive	Ponezumab	*IgG1	✓					Phase 2[a]
	Crenezumab	*IgG4	✓	✓		✓		Phase 3[a]
	Solanezumab	*IgG1	✓					Phase 3[a]
	Bapineuzumab	*IgG1	✓	✓			✓	Phase 3[a]
	Gantenerumab	**IgG1		✓		✓	✓	Phase 3[a]
	Aducanumab	**IgG1	Lower affinity	✓		✓	✓	Phase 4[a]
	Donanemab	*IgG1		✓			✓	FDA approved Phase 4[b]
	Lecanemab	*IgG1		✓	✓			FDA approved Phase 4[b]
Active	Amilomotide	Vaccines					✓	Phase 2/3[a]
	UB-311			✓		✓		Phase 2[a]
	AN1792						✓	Phase 2[a]
	ACI-24			✓		✓		Phase 2[b]
	ABvac40		✓	✓		✓		Phase 2[b]

*Humanized, **Human, [a]Terminated, [b]Ongoing.

The time-tested route for problem solving, i.e., from fundamental knowledge to application, has not been rigorously observed, where premature treatment efforts and resources have been spent with limited understanding of foundational concepts like amyloidosis, neuroinflammation, non-neuronal roles, vascular damage (including BBB disruption), the gut-brain axis, and overlapping pathologies. This fragmented approach has contributed to repeated drug development failures for AD, PD, and other amyloid diseases, underscoring the urgent need to rethink strategies for tackling neurodegeneration.

LLPS under the "condensation pathway" and β-barrel formation along the "deposition pathway" are two important processes in amyloid protein aggregation, including oligomerization. Both pathways are supported by a wealth of experimental and computational studies and may co-exist dynamically in silico, in vitro and in vivo. Although advancements in imaging, chromatography, mass spectroscopy, and computational modeling have greatly improved our understanding of early-stage amyloid protein aggregation, reconciling and integrating condensation and deposition pathways to delineate oligomerization and fibrillization remains a significant challenge. This complexity is heightened in cellular environments containing both phase-separated compartments and an aqueous continuum, presenting theoretical and experimental hurdles.

The existence and toxicity of β-barrel oligomers arising from the nucleation of full-length and fragmental amyloid proteins have been supported by experimental and computational data over the past two decades. Yet how well these β-barrels may represent the highly heterogeneous oligomeric populations in cell culture and in vivo, structurally and pathologically, is unknown. Are β-barrel oligomers entirely on-pathway or off-pathway in amyloidosis? If deemed on-pathway, how would these hybrid α-β secondary structures efficiently transform into cross-β amyloid architectures favored by energy minimization? If off-pathway, how would the production of β-barrel oligomers impede the kinetics of amyloid protein nucleation, elongation and saturation and seem to be largely absent over long incubations (days to weeks) under TEM? While scientifically arresting and essential, how to extrapolate the toxicities of small oligomers isolated in solvents[16,91] for assessing neurodegeneration elicited by an ever-changing "soup" of monomers, oligomers, protofibrils and amyloid fibrils?

The transient and polymorphic oligomeric states and their tendency and routes of initiating cytotoxicity are, in essence, determined by their inherent partial hydrophobicity and metastable free-energy minima. We believe a full understanding of the oligomers, from their occurrence (cradle) to their elicited cytotoxicity and neurodegeneration (grave) should not be attributed solely to the biophysical properties of amyloid proteins but be examined within a broader context where chaperone proteins, nucleic acids, small molecules, salts, solvent pH, cell membranes, as well as biological barriers may initiate interactions with amyloid proteins, individually or collectively, to impact cell function and homeostasis.

To date, determining oligomers' structural and toxicity profiles remains a key challenge, hindering the success of monoclonal antibody therapies in clinical trials for AD and other amyloid diseases like PD, ALS, or HTT (Table 1)[92]. The multifactorial nature of amyloid diseases further complicates treatment. AD pathogenesis, for example, involves not only Aβ oligomerization and plaque accumulation but also neurofibrillary tangles, neuroinflammation, and synaptic loss. Immunotherapies targeting Aβ plaques alone fail to address these interconnected factors, leading to the widespread failure of clinical trials[92]. The lack of animal models accurately replicating the complex pathology of amyloid diseases—beyond specific features like APP/PS1 or BACE1 mice for AD and αS or LRRK2 models for PD—further hinders progress in developing effective therapies.

Lecanemab, a humanized version of the murine mAb158 antibody co-developed by Eisai and Biogen, targets soluble Aβ oligomers and protofibrils. Its recent Phase 4 trial showed a reduction in Aβ levels in two-thirds of participants and slowed cognitive and functional decline in those with mild cognitive impairment and mild dementia[18]. Donanemab (Table 1), developed by Eli Lilly, targets the N-terminal truncated form of Aβ in amyloid plaques and removes plaques via microglial phagocytosis[93,94]. Donanemab has shown promising results in reducing amyloid levels and slowing disease progression in early-stage AD. Both lecanemab and donanemab, intravenously administered therapies, received accelerated approvals but require further evidence on long-term safety and therapeutic impact.

The molecular origin of APEL[22] stems from a collection of single oligomer/seed-VE cadherin interactions, the straw that breaks the camel's back in terms of endothelial leakiness on the microscopic scale. While no significant toxic effects have been identified to result from APEL, as observed with endothelial cell lines and mouse models exposed to the oligomers and seeds of Aβ and αS on the timescales of minutes to hours[22], the long-term implications of APEL warrant much exploration in the light of the high correlation between CAA and AD[19]. Hence, considering the systemic spread of amyloid proteins and bodily anatomy of the vasculature, we believe much research is needed to understand the implications of APEL and their mitigation for elucidating the paracellular transport of amyloid proteins and their associated

Table 2 | Major challenges for understanding oligomerization and neurotoxicity

Oligomer heterogeneity and toxicity	- What specific mechanisms underlie the toxic effects of amyloid oligomers on neurons, and how do these differ from the effects of larger aggregates like plaques?
	- How do the size, morphology, and structural properties of oligomers influence their toxicity?
	- How do PTMs impact oligomer interactions with cellular targets and their pathological effects?
Dynamic pathways	- How do LLPS, β-barrel formation and fibrillization intersect or diverge in vivo, and what are their relative contributions to amyloid aggregation and disease pathology?
	- Are β-barrel oligomers always on-pathway intermediates, or can they be off-pathway?
In vivo relevance and isolation	- How can native oligomers, present at low concentrations in CSF or plasma, be isolated and characterized reliably?
	- To what extent can synthetic oligomers be modified to mimic the structure and function of native species in vivo?
Disease modeling and detection	- How can disease models better replicate the complexity of in vivo environments to study amyloid aggregation and interactions with cellular and systemic factors?
	- What advanced techniques can improve the detection and characterization of oligomers in vivo, given their transient nature and low abundance?
Environmental interactions	- How do interactions with circulating proteins in biological environments affect oligomer stability, structural evolution, toxicity, and recognition by therapeutic agents?
	- What are the implications of the protein corona of oligomers and more advanced aggregates along the free-energy landscape rendered by environmental proteins, lipids, metals and ions?
	- What is the role of in vivo PTMs in modulating amyloid interactions with circulating proteins?
Therapeutic challenges	- How can future strategies overcome challenges in selectively disrupting pathological condensates without affecting normal phase separation, tuning LLPS dynamics to restore homeostasis, and achieving sub-stoichiometric inhibition?
	- Why have immunotherapies targeting amyloid species largely failed in clinical trials, despite promising preclinical results?
	- How can therapeutic strategies for neurodegenerative diseases be designed to address the multifaceted nature of their pathogeneses, including oligomer toxicity, dysregulated neuronal-glial crosstalk, vascular permeability, and synaptic dysfunction?
APEL	- Does APEL facilitate the cross-talks between the pathologies of AD, PD, and other brain and systemic amyloid diseases?
	- Can APEL mitigation yield therapeutic outcomes against amyloid diseases?

BOX 1

Envisioned directions for oligomer research

(1) In Vivo PTMs: a growing body of evidence supports the notion that PTMs are present across an array of amyloid proteins[96]. Future research should identify in vivo PTMs that promote or inhibit LLPS and β-barrel formation for major amyloid proteins and investigate how different stimuli regulate their LLPS and β-barrel formation. Additionally, the cross-talks between disease-relevant PTMs warrant investigation.

(2) The protein corona: physical interactions also play an indispensable role in complex biological environments to form an amyloid corona[97,98]. Apart from influencing the structural evolution and pathological footprint of amyloid aggregates in vivo, this corona could also hinder recognition of the oligomers by their antibodies or drugs developed in vitro. Understanding these transformations is essential for grasping how amyloid protein toxicity evolves during aggregation and recognizing the limitations of in vitro systems in mimicking the complex protein environment and toxicity in vivo.

(3) Disease modeling: future disease modeling should: (1) identify key in vivo proteins that interact with amyloid protein aggregates of changing hydrophobicity and influence their accessibility and interactions with cell receptors, enzymes, or immune cells, (2) consider the role of PTMs in modulating in vivo amyloid protein aggregation and toxicity, and (3) develop high-throughput proteomics and machine learning for examining protein aggregation and their cellular interactions in vivo.

(4) Drug delivery: nanocomposites could be tailor-designed for enhancing the efficacy, stability and delivery of anti-neurodegenerative antibodies. Nanoparticles can sequester amyloid proteins through the corona effect and disrupt amyloidosis by outcompeting protein-protein interactions. Nanoparticles synthesized via polymerization-induced self-assembly (PISA)[99] may find use for targeting oligomers and protofibrils by adjusting their amphiphilicity and incorporating amyloidogenic motifs for improved targeting.

(5) Multifactorial targeting: accumulative evidence points to multifaceted mechanisms for neurodegeneration, intrinsically linked to the physicochemical and structural properties of oligomers. Interactions of oligomers with glial cells can provoke neuroinflammation and modulate microglial phenotypic alterations, further amplifying the toxic milieu[100]. Collectively, the complexity of oligomer toxicity underscores the need for multimodal inhibitors.

biomolecules, cerebrovascular damage, blood vessel wall infiltration[95], and neurotransmission impairment in AD, PD and other neurodegenerative disorders. In connection with these aspects, mitigating APEL may yield surprisingly effective outcomes in treating these diseases.

Table 2 summarizes the major challenges for current research on oligomerization and their entailed neurodegeneration and vascular integrity. Subsequently, Box 1 provides our envisioned directions for the field of oligomer research moving forward.

The effects of amyloid protein co-aggregation and cross seeding[1] on oligomerization and their elicited toxicity, including neurotoxicity, remain a largely uncharted territory and are beyond the scope of this presentation. Furthermore, towards therapeutics, innovative strategies aimed at mitigating the toxicity of oligomers (including protofibrils) need to be explored utilizing in vitro, in vivo and ex vivo (e.g., organoids) models and fulfilling the promise of molecular assembly, polymer chemistry, nanomedicine, gene technology, -omics (proteomics, metabolomics and transcriptomics), and machine learning. The complexity, knowledge gaps, and potential convergence in the structure and activity of oligomers throughout their lifecycle remain to be fully resolved. Addressing these challenges is crucial for enabling the bottom-up design of future therapeutics targeting neurological and metabolic amyloid diseases.

Data availability

All the data supporting the study are available within the Perspective.

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Author contributions

P.C.K. conceived the manuscript and wrote the "Abstract" and "Introduction". P.C.K. and F.D. supervised drafting of the manuscript and its revisions. P.C.K., N.A. and S.K. wrote the "Outlook". H.T., Y.S., F.D., N.A. and Y.L. wrote the main section of "Protein aggregation, neurodegeneration, and endothelial leakiness". H.T., N.A., S.K. and P.C.K. proofread the manuscript and its revisions. H.T., N.A. and Y.L. prepared the figures and copyright forms.

Competing interests

The authors declare no competing interests.

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