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Design and Molecular Dynamics Simulation of Peptide Inhibitors Targeting Amyloid-Beta Aggregation

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ABSTRACT

Background: Aggregation of amyloid-beta (Abeta) into oligomers and fibrils is central to Alzheimer's disease pathogenesis, and peptide-based ligands remain among the most structurally precise tools for blocking this process. Objective: To synthesise published literature on rationally designed and computationally identified peptide inhibitors of Abeta aggregation and the molecular dynamics (MD) studies that explain their mechanisms. Methods: Narrative review of peptide-inhibitor design and MD simulation studies targeting Abeta aggregation, identified via PubMed, Google Scholar, Crossref and bioRxiv. Findings: Recognition-sequence peptides (KLVFF), beta-sheet breakers (LPFFD), N-methylated and D-enantiomeric peptides, molecular tweezers (CLR01) and cell-penetrating designs each interfere with the central hydrophobic core or C-terminal self-recognition motifs; MD and steered-MD studies, informed by solid-state NMR and cryo-EM fibril structures, converge on disruption of in-register beta-sheet stacking and salt-bridge networks as a shared mechanism. Conclusions: Peptide inhibitors targeting defined Abeta interfaces are mechanistically well characterised, but proteolytic instability and blood-brain barrier penetration remain the principal translational barriers.

Keywords: *amyloid-beta; peptide inhibitor; molecular dynamics simulation; Alzheimer's disease; aggregation inhibition*

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Abstract

Background: Aggregation of amyloid-β (Aβ) into oligomers and fibrils is central to Alzheimer's disease pathogenesis, and peptide-based ligands remain among the most structurally precise tools for blocking this process. **Objective:** To synthesise published literature on rationally designed and computationally identified peptide inhibitors of Aβ aggregation and the molecular dynamics (MD) studies that explain their mechanisms. **Methods:** Narrative review of peptide-inhibitor design and MD simulation studies targeting Aβ aggregation, identified via PubMed, Google Scholar, Crossref and bioRxiv. **Findings:** Recognition-sequence peptides (KLVFF), β-sheet breakers (LPFFD), N-methylated and D-enantiomeric peptides, molecular tweezers (CLR01) and cell-penetrating designs each interfere with the central hydrophobic core or C-terminal self-recognition motifs; MD and steered-MD studies, informed by solid-state NMR and cryo-EM fibril structures, converge on disruption of in-register β-sheet stacking and salt-bridge networks as a shared mechanism. **Conclusions:** Peptide inhibitors targeting defined Aβ interfaces are mechanistically well characterised, but proteolytic instability and blood-brain barrier penetration remain the principal translational barriers.

Keywords: amyloid-beta; peptide inhibitor; molecular dynamics simulation; Alzheimer's disease; aggregation inhibition

Introduction

Alzheimer's disease is characterised neuropathologically by extracellular deposition of amyloid-β (Aβ) peptides, generated by sequential β- and γ-secretase cleavage of the amyloid precursor protein, and the amyloid cascade hypothesis has for three decades framed Aβ misfolding and aggregation as an early driver of downstream neurodegeneration [1]. Aβ monomers self-assemble through nucleation-dependent pathways into soluble oligomers, protofibrils and cross-β fibrils, with oligomeric species now considered principal mediators of synaptotoxicity [2,3]. Because aggregation proceeds through sequence-specific, structurally defined intermolecular contacts, short peptides that mimic or block these contacts have long been pursued as aggregation inhibitors, complementing small-molecule and antibody approaches. Molecular dynamics (MD) simulation, increasingly informed by high-resolution fibril structures, has become the principal computational tool for rationalising how such peptides

engage Aβ and for guiding new designs [4]. This review synthesises the published record on peptide inhibitor design and the MD studies that illuminate their inhibitory mechanisms.

Methods

This is a narrative review of published peptide inhibitor design and molecular dynamics simulation studies targeting amyloid-beta aggregation. Relevant primary and review articles were identified through structured searches of PubMed, Google Scholar, Crossref and bioRxiv using combinations of the terms "amyloid-beta", "peptide inhibitor", "aggregation", "fibril", "molecular dynamics" and "Alzheimer's disease", supplemented by citation chaining from key papers. Studies were included if they reported an experimentally or computationally characterised peptide-based Aβ aggregation inhibitor, an atomic-resolution Aβ fibril structure used to rationalise inhibitor design, or an MD/steered-MD analysis of an inhibitor-Aβ complex; each reference was individually verified against its indexed record before inclusion. No new simulations, docking runs or wet-laboratory experiments were performed for this article; all sequences, structures and findings reported below are drawn directly from the cited literature.

Amyloid-Beta Aggregation Mechanism and Druggable Interfaces

High-resolution structures of Aβ42 fibrils solved by solid-state NMR and cryo-electron microscopy show parallel, in-register cross-β sheets stabilised by a hydrophobic core spanning residues 17-42, a salt bridge between Lys28 and the C-terminal carboxylate, and a hairpin turn around residues 22-29 [5,6,7,8]. These structures converge on two recurring druggable features: the central hydrophobic cluster (Leu17-Ala21, containing the KLVFF recognition sequence) and the C-terminal segment that mediates lateral fibril packing. The self-recognition property of the KLVFF motif — its ability to bind full-length Aβ through the same sequence it occupies within the fibril — was first exploited by Tjernberg and colleagues, who showed that a KLVFF-based pentapeptide arrests fibril formation by capping growing aggregates rather than by generic surface adsorption [9]. Structure-function analyses subsequently mapped which KLVFF-flanking residues and charge modifications preserve inhibitory potency [10]. Because nucleation and elongation depend on cooperative, multivalent β-sheet contacts, inhibitors need not achieve high-affinity single-site binding; instead, effective designs sterically or electrostatically disrupt the register of β-strand stacking, providing multiple independent, druggable strategies converging on the same aggregation pathway [11].

Rationally Designed and Computationally Identified Peptide Inhibitors

Beyond the founding KLVFF pentapeptide, several complementary peptide chemistries have been developed. β-sheet breaker peptides such as LPFFD, in which a proline substitution disrupts extended β-conformation while retaining Aβ-recognition residues, reduced amyloid burden in a rat model of cerebral amyloidosis [12]. N-methylation of backbone amides along a KLVFF-derived scaffold blocks the hydrogen-bonding register required for β-sheet extension, and iterative optimisation of methylation position and flanking charge yielded inhibitors active at sub-stoichiometric ratios [13]. Mirror-image phage display against D-Aβ42 identified the all-D-peptide D3, which binds natural L-Aβ, is intrinsically protease-resistant, reduces oligomer load, and improves pathology and behaviour in transgenic mice after oral administration; subsequent optimisation improved its oligomer-elimination potency [14,15,16]. D-amino-acid pseudopeptide and non-natural residue substitutions have similarly been used to combine Aβ affinity with proteolytic stability [17]. Structure-guided approaches have used fibril and oligomer coordinates directly as templates: Lu and colleagues designed peptide inhibitors from segments identified in Aβ fibril structures [18], Horsley and colleagues rationally engineered a macrocyclic peptide inhibitor of Aβ42 fibril formation and toxicity [19], and cell-penetrating peptide conjugates were designed to simultaneously block extracellular aggregation and intracellular toxicity [20]. Computational pipelines have also generated candidates de novo: Eskici and Gur mutated a validated reference peptide combinatorially and used docking followed by steered MD to prioritise three lead sequences with improved predicted binding free energy [21], while self-assembling hexapeptides were computationally screened for complementary binding to the Aβ hydrophobic core [22]. Non-peptidic molecular tweezers (CLR01), which bind lysine and arginine side chains, act through an analogous process-specific rather than sequence-specific mechanism and are included here as a mechanistic comparator [23]. Hybrid peptide-polyphenol conjugates (KLVFF-EGCG) combine recognition-driven targeting with polyphenol-mediated remodelling of oligomers [24].

Table 1: Atomic-resolution Aβ42 fibril structures used as templates for structure-guided peptide inhibitor design.

Structure/method	Year	Key structural feature	Reference
ssNMR, Aβ(1-42) fibril	2005	Parallel in-register β-sheets; hairpin turn 22-29	[5]
ssNMR, monomorphic Aβ42 fibril	2016	Atomic-resolution triple-β-motif protofilament fold	[6]
ssNMR, disease-relevant Aβ(1-42) fibril	2016	S-shaped protofilament with defined salt bridge network	[7]
Cryo-EM, Aβ(1-42) fibril	2017	Near-atomic resolution LS-shaped fibril polymorph	[8]

Figure 1: Structural interfaces of the Aβ42 fibril targeted by the peptide inhibitor classes discussed in this review, tallied by the number of cited inhibitor classes reported to engage each interface, based on solid-state NMR and cryo-EM fibril structures and the associated design literature [5,6,7,8,9,12,13,14,23].

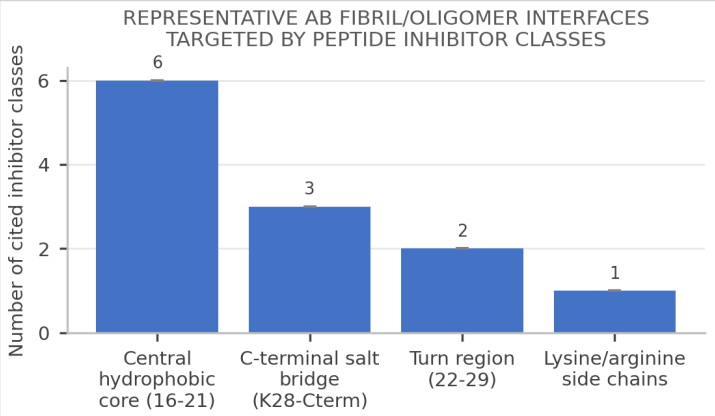
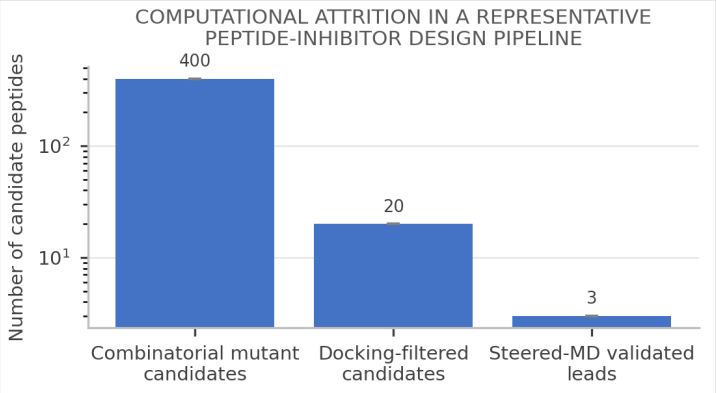


Figure 2: Attrition of candidate peptides through a representative combinatorial-mutation, docking and steered molecular dynamics design pipeline, illustrative of the workflow reported for computationally identified Aβ peptide inhibitors [21,22].



Molecular Dynamics Insights into Inhibition Mechanisms

MD simulation has been essential for interpreting how these peptides act once bound. All-atom and steered-MD studies of KLVFF and its analogues bound to Aβ fibril or oligomer models show that inhibitory peptides preferentially dock antiparallel to the native in-register strand, competitively occluding the same interface used for monomer addition and thereby capping fibril growth rather than dissolving existing aggregates [25,26]. Comparative simulations of designed hexapeptides indicate that inhibitory potency correlates with the number and persistence of hydrogen bonds and hydrophobic contacts formed with the central hydrophobic cluster, and that steric mismatch introduced by D-residues or N-methylation destabilises the parallel β-sheet register even when overall binding affinity is comparable to non-inhibitory ligands [22,17]. Broader reviews of Aβ/drug interaction simulations emphasise that oligomeric, rather than fibrillar, Aβ assemblies are more conformationally heterogeneous targets, so inhibitor-bound trajectories must be interpreted as ensembles rather than single poses, and that computational estimates of binding free energy (for example, via MM-GBSA or free-energy perturbation) require experimental calibration against ThT-based aggregation kinetics [4]. Collectively, these simulations support a shared mechanistic model in which effective peptide inhibitors intercept a small number of structurally conserved recognition interfaces rather than acting through generic surface coverage.

Translational Challenges: Stability, Delivery, and Selectivity

Despite mechanistic clarity, peptide inhibitors of Aβ aggregation face substantial translational hurdles. Natural L-peptides such as KLVFF derivatives are rapidly degraded by serum and brain proteases, motivating D-amino-acid substitution, N-methylation and macrocyclisation strategies that improve stability without necessarily preserving potency [17,13,19]. Even protease-resistant D-peptides such as D3 must cross the blood-brain barrier at sufficient concentration and duration to engage brain Aβ pools, and pharmacokinetic and oral-bioavailability data for D3 illustrate both the feasibility and the dosing burden of this approach [15]. Cell-penetrating peptide conjugation offers one route to intracellular and membrane-associated Aβ pools but introduces additional considerations of off-target membrane activity and manufacturing complexity [20]. Recent reviews highlight AI-guided sequence optimisation and delivery-vehicle engineering (nanoparticle or conjugate-based) as active strategies for reconciling potency, selectivity for pathological over physiological Aβ conformers, stability and CNS bioavailability [27,28].

Table 2: Representative peptide-based inhibitors of Aβ aggregation, their design strategy, and principal reported finding.

Peptide / class	Design strategy	Principal reported finding	Key reference
KLVFF pentapeptide	Native recognition-sequence fragment	Arrests Aβ fibril formation by capping growing aggregates	[9]
LPFFD	Proline-substituted β-sheet breaker	Reduces amyloid burden in a rat model of cerebral amyloidosis	[12]
N-methylated KLVFF analogues	Backbone N-methylation to block β-sheet H-bonding	Sub-stoichiometric inhibition of aggregation and toxicity	[13]
D3 (all-D peptide)	Mirror-image phage display against D-Aβ42	Protease-resistant; reduces oligomer load and improves pathology in transgenic mice	[14,15,16]
CLR01 molecular tweezer	Lysine/arginine-selective supramolecular ligand	Broad-spectrum, process-specific inhibition of amyloid assembly and toxicity	[23]
Cell-penetrating peptide conjugates	CPP fusion for intracellular/extracellular targeting	Inhibits both extracellular aggregation and intracellular cytotoxicity	[20]

Conclusion

Published structural, design and MD literature converges on a coherent picture: Aβ aggregation proceeds through a limited number of structurally defined, druggable interfaces, and peptide inhibitors spanning KLVFF-derived recognition sequences, β-sheet breakers, N-methylated and D-enantiomeric peptides, and cell-penetrating designs each interrupt these interfaces by mechanisms that MD simulation has helped resolve at atomic resolution. The principal barrier to clinical translation is no longer mechanistic understanding but pharmaceutical optimisation — proteolytic stability, blood-brain barrier delivery and selectivity for pathological Aβ species — that future structure-guided and computationally assisted design efforts will need to address.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Novotny S conceived the review, conducted the literature search, and drafted the manuscript. Kazlauskas R contributed to the literature screening and technical framing and critically revised the manuscript for important intellectual content. Laine M contributed additional context and approved the final version for submission.

Ethics Statement

Not applicable. This is a narrative review of previously published literature and did not involve new experimental data, human participants, or animal subjects.

Data Availability

No new data were generated or analyzed for this review. All sources synthesized are cited in the reference list and are available through their original publishers.

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