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Understanding familial Alzheimer’s disease: The FIST mechanism of γ-secretase

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Abstract

Understanding Alzheimer's disease is a central challenge of the 21st century, as the disease affects tens of millions of people and kills a million people each year, with current drugs having modest effect. This paper reviews how computational science integrating new cryo-electron microscopy structures and biochemical and clinical data has led to a causative model of familial Alzheimer's disease (fAD). The model's basis is open and compact conformational states of the membrane protease γ-secretase, controlled by transmembrane helix "fingers" that hold the substrate either tightly or loosely. The two states are in thermal equilibrium and lead to different amounts of long and short Aβ peptides, explaining the much-debated Aβ42/Aβ40 ratio. Pathogenic mutations shift the equilibrium towards the open state by reducing the stability and hydrophobic packing of the enzyme-substrate complex, which increases toxic Aβ42 and other longer peptide forms compared to Aβ40. In contrast, drugs that selectively target longer, pathogenic Aβ peptides should preferentially stabilize the compact state to reverse this tendency. The model may explain how inherited mutations cause fAD and provides a molecular roadmap for the development of γ-secretase modulators, one of the most promising causative treatment strategies in current Alzheimer research. In summary, we showcase the power of modern multiscale computational science in integrating biochemical, protein-structural and clinical data to elucidate complex disease mechanisms.

Graphical/Visual Abstract and Caption

Computer modelling has recently been instrumental in integrating a wide range of complex biochemical, clinical, and protein-structural data into a unified model of how mutations cause familial Alzheimer's disease, and how drugs can be tailored to target specific pathogenic conformations of the protein γ-secretase.
1. INTRODUCTION

The present paper summarizes how multiscale computational science has integrated complex data such as membrane protein structures, clinical data of patients carrying pathogenic protein variants, and biochemical assays, into a causative model, the fit-stay-trim (FIST) model of γ-secretase function, which can explain how inherited mutations cause familial Alzheimer's disease (fAD) and how successful drugs should delay disease by protein-conformational-selective molecular action.

Alzheimer's disease (AD) is suffered by tens of millions of people world-wide and treatments remain, after decades of intense research, mainly symptomatic and only modestly able to delay disease progression.\textsuperscript{1–3} The lack of progress is largely believed to be due to the complex etiology of the disease, with many risk modifiers, a broad clinical spectrum, and diverse biochemical pathways contributing to diseases, emphasizing the need for molecular-based etiology.\textsuperscript{4–8} Although many hypotheses of AD exist, the amyloid hypothesis has dominated efforts to identify more efficient causative treatments: This hypothesis assumes that β-amyloid (Aβ) peptides in brains, probably in the form of toxic intracellular oligomers, are the main cause of the disease; the exact mode of pathogenesis and how the production of Aβ relates to disease remains unclear.\textsuperscript{9–12}

AD comes in two forms: Early-onset fAD and late-onset sporadic AD (sAD). fAD typically emerges between 30-65 years, whereas sAD symptoms typically occur after 65 years.\textsuperscript{13,14} fAD is genetically linked to mutations in the presenilin-1 (PS1) gene,\textsuperscript{15–17} presenilin-2 (PS2) gene,\textsuperscript{18} and amyloid-β precursor protein (APP) gene.\textsuperscript{19,20} Although fAD is a small subset of total AD, it forms the basis for much mechanistic research due to the direct genotype-phenotype link.\textsuperscript{21,22} PS1 mutations are the most common cause of fAD, and also more severe: The average age of onset seems significantly higher for APP than PS1 mutations,\textsuperscript{23} thus making PS1 of particular interest as the main causal basis for understanding the disease,\textsuperscript{24} and the basis of the computational and experimental work reviewed in this paper.
2. γ-SECRETASE AND Aβ

Aβ peptides are produced by γ-secretase (Figure 1), a four-subunit intramembrane aspartyl protease that has PS1/PS2 as its multipass transmembrane catalytic subunit. The β-amyloid precursor protein (APP) is initially cleaved by β-secretase (Figure 1a), producing the C-terminal fragment APP-C99 which is then cleaved in consecutive 3-amino acid steps along two pathways (Figure 1b-1c): Aβ49 → Aβ46 → Aβ43 → Aβ40 → Aβ37 and Aβ48 → Aβ45 → Aβ42 → Aβ38 by γ-secretase, which leads to Aβ peptides of different lengths (Figure 1d), with Aβ40 being the dominating product; the physiological significance of these cleavage products remains poorly understood. Possible roles include an unknown function in the synaptic cleft, being anti-microbial peptides, reducing the activation of potassium channels, neuroprotection, preventing blood-brain-barrier leaks and suppressing cancer via inhibiting oncogenic viruses. However, the most probable role seems to be in memory improvement at normal Aβ levels although the exact mechanism remains unknown. The catalytic pore of PS1 inside the membrane where the substrate binds contains substrate-binding cavities (sometimes called S1’, S2’, and S3’) that help to place the substrate and orient it towards the catalytic aspartates during peptide bond cleavage.
In addition to PS1/PS2, γ-secretase includes the other subunits presenilin enhancer protein 2 (PEN-2), anterior-pharynx-defective protein 1 (APH-1) and nicastrin (NCT; Figure 2a) which contribute to the overall stability and function of the protein.\textsuperscript{37–39} PS1 consists of nine transmembrane helices (TM1-TM9) and a large hydrophilic loop between TM6 and TM7.\textsuperscript{40} Two aspartate residues in the membrane catalytic site of PS1 are required for cleavage of the peptide bonds in APP-C\textsubscript{99} that leads to amyloids (Figure 2b) but also endoproteolytic maturation of PS1 itself, by breaking the large hydrophilic loop to produce N- and C-terminal PS1 fragments (NTF and CTF, Figure 2c).\textsuperscript{41} The structure and function of γ-secretase has been excellently reviewed elsewhere.\textsuperscript{25,42–46}

Figure 2. Structure and function of γ-secretase and fAD mutations. (a) γ-secretase structure (PDB code 6IYC\textsuperscript{47}) in a membrane model. (b) Cleavage of APP by β-secretase followed by γ-secretase to form Aβ peptides. (c) Topology of presenilin showing TM1-9, the two catalytic aspartate residues (D), the hydrophilic loop, and maturation cleavage. (d) Diverse phenotypes of fAD mutations. (e) Correlation between Aβ\textsubscript{42}/Aβ\textsubscript{40} ratio and average age of symptom onset (adapted from Figure 5 in Sun et al.\textsuperscript{48} - no significance). (f) Reanalysis data adapted from Figure 1 in Tang et al.\textsuperscript{49} Removal of one outlier results gives significant correlation.
Aβ has been suggested to cause disease by a direct mode of action, such as interaction with cell membranes after its formation (Figure 2d).\textsuperscript{50–52} However, total Aβ production often decreases upon fAD mutation,\textsuperscript{48,53} implying that Aβ overload does not cause disease by itself.\textsuperscript{11} Consistent with this, therapeutic inhibition of γ-secretase has not been successful.\textsuperscript{5,11,48} The amyloid hypothesis\textsuperscript{12} has accordingly shifted to an emphasis on the relative amount of Aβ\textsubscript{42} or modified versions that include e.g. metal ions.\textsuperscript{55–59} Indeed, most fAD mutations, often with overall loss of activity, tend to increase the relative amount of longer Aβ peptides, e.g. the Aβ\textsubscript{43}/Aβ\textsubscript{40} ratio, although not always.\textsuperscript{48,60} More generally, mutations tend to impair the cleavage efficiency of the enzyme.\textsuperscript{48,61} The N-terminal half of Aβ is hydrophilic, but the cleaved C-terminal part is hydrophobic, and thus Aβ\textsubscript{42} is more hydrophobic and aggregation-prone than Aβ\textsubscript{40},\textsuperscript{62} and senile plaques feature relatively more long Aβ.\textsuperscript{53} We note that monomeric Aβ\textsubscript{40} seems important for normal brain function\textsuperscript{5,64–66} which could suggest that nondifferentiated Aβ clearance is not desirable, and indeed apparently not very clinically effective.\textsuperscript{57,68}

Although Aβ\textsubscript{42} is typically considered the primary amyloidogenic species, Aβ\textsubscript{43} also plays an important role (Figure 1d)\textsuperscript{69–72}: It is perhaps even more aggregation-prone and toxic\textsuperscript{69}, and some pathogenic PS1 mutations such as R278I and L435F produce substantial amounts of Aβ\textsubscript{43}.\textsuperscript{69–71}

Early seminal work found that fAD mutations induce conformational changes in PS1 that may be functionally important, with a tendency to produce a more "closed" structure as measured by fluorescence lifetime imaging of the distance between the N- and C-termini of the protein,\textsuperscript{73} later confirmed by similar studies.\textsuperscript{74} These studies provided evidence of conformational effects of mutants as key to a molecular understanding of fAD, as also seen from cysteine mutation studies\textsuperscript{75} and photo-crosslinkable amino acid studies,\textsuperscript{76} and with electron microscopy providing the structural support for the multi-conformational nature of the protein\textsuperscript{77–80}.

Importantly, the names "open" and "close" used to designate N- and C-termini distances should not be confused with the open and close states discussed here, which relate to the conformational space available in the catalytic pocket controlled by the tilting and relative positions of PS1 TMs. The "open" conformation from fluorescence lifetime imaging of the N- and C-termini approximately corresponds to the "semi-open" conformation of the substrate binding site, and the closed (in terms of N- and C termini) corresponds to the open state in terms of the substrate cavity: The mature (auto-cleaved) open state in the membrane has its N- and C-termini closer together due to a tilting of the TMs away from parallel upon auto-maturation\textsuperscript{81,82} (presumably also why mutants in
the immature state typically (but not always) display strongly impaired activity and reduced processivity\textsuperscript{72} – its autocleavage produces these two states allowing the non-parallel TM displacement)\textsuperscript{83}. Expansion in the membrane-residing substrate binding sites has been seen consistently in a number of simulations of both PS1 and PS2,\textsuperscript{84–88} and with a handful of fAD mutants by direct structural simulation,\textsuperscript{86} as discussed below. Thus, in all the following, open, semi-open, and compact/closed refer exclusively to the membrane protein conformations and active site space.

A main finding inspiring the FIST model was the 2013 work by Okochi et al., who interpreted the cell data in a kinetic model where PS1 mutations lead to reduced catalytic turnover (k\textsubscript{cat}) and faster release of the substrates (k\textsubscript{s}) to the effect of reduced trimming, and drugs that lower the A\textsubscript{β42}/A\textsubscript{β40} ratio do the opposite.\textsuperscript{89} While substrate affinity and enzyme-substrate stability was not directly emphasized, it can be considered implied by a standard Michaelis-Menten framework where stronger affinity (lower Michalis constant, K\textsubscript{M}) leads to higher overall turnover (k\textsubscript{cat}/K\textsubscript{M}). Since this aspartyl protease is notoriously slow (estimated in 2015 as k\textsubscript{cat} \sim 0.0012 s\textsuperscript{-1} and K\textsubscript{M} \sim 0.2 mM, giving k\textsubscript{cat}/K\textsubscript{M} \sim 6 s\textsuperscript{-1} M\textsuperscript{-1})\textsuperscript{90} even marginal effects of mutations on this speed could be problematic. The impaired processing towards increased A\textsubscript{β42}/A\textsubscript{β40} ratio being caused by reduced trimming activity was supported by other data soon after.\textsuperscript{91}

3. CHEMISTRY DEFINES CLINICAL SEVERITY OF PS1 MUTATIONS

The clinical data of the PS1 variants is the ultimate endpoint of any model of disease, and any molecular mechanism and etiology should ideally explain the diverse outcomes of different PS1 variants. More than 200 fAD-related PS1 variants have been reported\textsuperscript{92,93} that inflict heterogeneous clinical phenotypes (Figure 2d).\textsuperscript{94,95} Perhaps surprisingly but importantly, the chemical nature of the mutation (its chemical properties and effect on the substrate cleavage) explains a large part of the variation in the clinical age of symptom onset, which partly relates to the strongly penetrant monogenic nature of PS1-related fAD. Yet as expected even for fAD (and much more so for milder sAD, which is more sensitive to risk modifiers) considerable noise exists, even within the families carrying the same mutant.\textsuperscript{23} Age of onset is also affected by associated presence of the APOE ε4 isoform.\textsuperscript{96,97} Despite these confounders, the average age of symptom onset, when taken as an
indicator of clinical severity, has been shown to be an important benchmark for understanding the geno-type-phenotype relations and the molecular mechanism more completely.23,48,60

The first step towards a molecular structure-based understanding of both the biochemical data summarized by the kinetic model of Okochi et al.89 and the clinical data summarized e.g. by Ryman et al.23 was made in 2016, using chemoinformatic models to identify correlations between the amino acid properties of the PS1 substitutions and their clinical impact, measured by age of symptom onset.60 We note that the clinical data for fAD are quite noisy: Same-variant carriers even within the same family can display variations in age of onset >5 years and in some cases much more.54,60 However, for a strongly penetrant monogenic disease as fAD, we hypothesized that such relationships are key to a complete etiology of fAD with translational relevance, with average values from multiple patients being a useful test of a theory since many such data are available from clinical databases and reviews23. The analysis importantly found that the mutations that produce large Aβ42/Aβ40 ratios coincided with loss of thermodynamic stability and hydrophobic packing as computed with protein stability methods and amino acids property scales.60 This finding motivated the idea that the enzyme-complex stability and hydrophobic packing within the membrane controls the kinetics of the APP-C99 cleavage and Aβ production summarized by Okochi et al.89

The year after, Sun et al. performed a systemic investigation of 138 PS1 mutations linked to AD (Figure 2e), by far the most complete dataset of PS1 mutations.48 Though the authors analysed Aβ42 and Aβ40 production, Aβ43 analysis was missing, which could also affect age of onset correlations for at least some of these mutants.69–71 Perhaps also of note, Cacquevel et al. observed that the purified form of γ-secretase is more sensitive to loss of activity upon PS1 mutation, which could also affect results.98

The Aβ production observed by Sun et al.48 was related to the reported clinical age of onset, as also done by other groups.60,99–101 The large majority of mutations are hypomorphic as one could expect,5 i.e. they lower enzyme activity and thus decrease formation of both Aβ42 and Aβ40 as compared to the wild-type, but interestingly associated with elevated Aβ42/Aβ40 ratios. The authors however found no significant relationship between the Aβ42/Aβ40 ratios and mean age of onset (Figure 2e).

In addition to the noise in clinical data and the special care need in terms of e.g. outlier analysis and confounders, some assayed data should be cautiously interpreted as they approach
zero enzyme activity (detection limit), and Aβ42/Aβ40 ratios become uncertain as Aβ40 levels approach zero. Re-examination\(^4^9\) of the data by Sun et al.\(^4^8\) (Figure 2f) using several clinical data sets indicated a highly significant correlation (R\(^2\) = 0.12, p-value = 0.0011) between Aβ42/Aβ40 ratios and age of onset after removing the extreme outlier G384A, which displays very low activity probably (and thus a very small, uncertainty-increasing denominator in the Aβ42/Aβ40 ratio) because it is located very close to the substrate cleavage site.\(^4^9\) Thus, the genotype-phenotype relationship, our most important basis for a mechanistic understanding of fAD, is intact, and thereby, also the implication that the stability and hydrophobic packing is the central molecular determinant of both the cleavage process, the Aβ42/Aβ40 ratio, and the clinical endpoint, of course within the limit afforded by risk confounders.

Still, a complete structural confirmation of these clinical, chemical, and biochemical data remained elusive until the arrival of full enzyme-substrate structures in 2019, and subsequent molecular dynamics simulations of the associated membrane conformations of these complexes at physiological temperature.

4. MOLECULAR SIMULATIONS OF γ-SECRETASE

Cryo-electron microscopy (cryo-EM) experiences a revolution in terms of sample control and resolution.\(^1^0^2,1^0^3\) With the advent of cryo-electron microscopy structures of γ-secretase,\(^4^7,7^7^-^8^0,1^0^4\) many computational simulation studies were undertaken to understand the mechanism of the protein and the function of drugs directed towards it.\(^1^4,8^1,1^0^9^-^1^1^2,8^4^-^8^8,1^0^5^-^1^0^8\)

Kong et al. performed the first membrane-embedded simulation of PS1 (without maturation loop) in 2015 using a homology model derived from a non-human ortholog, observing important gate movements in TM2 and TM6 and TM9 that are now known to be functionally important.\(^1^1^3\) We note that the maturation loop has not been resolved in the experimental cryo-EM structures due to the extreme flexibility of this part. To understand why maturation is required for catalysis, we simulated human PS1 with the maturation loop in both the immature and mature catalytic active form, also in the membrane.\(^8^3\) Comparison of the mature and non-mature PS1 suggested that the loop dynamics are directly implicated in the gating mechanism, as also shown by Li et al.\(^1^1^4\) Conformational variations in the maturation loop connecting TM6 and TM7 led us to suggest that the loop acts as a “plug” that need to be unplugged by autoproteolysis in order to access the protein’s interior and the catalytic site.\(^8^3\)
Further investigation of the PS1 maturation loop motion revealed three distinct conformational states of PS1 and indicated that the mature loop regulates these motions. These three conformers differ near the two catalytic aspartates and in the C-terminal part of TM2 and N-terminal part of TM3. Molecular docking of Aβ42 and Aβ40 indicated best fit to the semi-open conformation state, and this was proposed to cause longer substrate residence time and thus more processing, giving shorter Aβ. The semi-open state was hypothesized to be the normal active conformer, whereas the closed and open states caused inactivity (too compact for substrate binding) or longer peptides (longer Asp-Asp distance, looser fit, earlier product release). These findings led to a model of γ-secretase function implying that the semi-open conformation should be preserved while the open state should be selectively reduced by therapeutic strategies.

The first all-atom MD simulation study of the full APP-C99 model within the most complete γ-secretase (except N-terminal loop, missing in experimental structures) was reported recently. The simulations were performed for both the apoform of γ-secretase and APP-C99-γ-secretase complex in a membrane. Using again the distance between catalytic aspartates as functionally relevant descriptor, two distinct conformational states of APP-C99 were found, one open (loose) and one closed (compact). The more open conformation was suggested to enable both cleavage pathways (imprecise fit) and earlier release of longer Aβ. All the experimental structures used as a basis for these dynamics were derived by cryo-EM at low (perhaps ~85 K) temperature. Thus, the simulations reflect dynamics at room temperature and complement the experimental structural data for APP-C83-γ-secretase complex. The distance between the two catalytic aspartates correlated with motions in TM2, TM6 and TM9.

Building on the chemoinformatic findings that stability and hydrophobic packing control substrate processing kinetics proposed by Okochi et al., these various findings were formalized in the FIST (fit-induce-stay-trim) model (Figure 3), where the TMs (2, 3, 6, 7, 9) are the “fingers” of the fist that “squeeze” the substrate to longer and less precise (along both cleavage pathways) or shorter cleavage products, depending on whether squeezing is loose or tight. The observation that an open and semi-open state (Figure 3a, 3b) with respect to substrate binding exist in equilibrium supports the argument that APP-C99 binding affinity may control cleavage and Aβ length. The model explains the function of γ-secretase modulators (GSMs) (Figure 3c, 3d) as favoring the compact state and increasing trimming (slow dissociation in the proposal by Okochi et al.) and fAD mutations as...
favoring the looser state (fast dissociation) leading to catalytic impairment and relatively more of the longer Aβ peptides (Figure 3e).  

Figure 3. The FIST (fit-stay-trim) model. (a) Room-temperature MD simulations based on the cryo-EM coordinates revealed two major conformations, compact and open, defined by the distance between the two catalytic aspartates (D). (b) Apo-protein simulations found three main conformations, closed, semi-open and open. (c) QSAR indicates the importance of binding energy, dehydration, hydrogen-bond optimization, and topology (length vs. size). (d) GSMs contribute their binding affinity to stabilize the γ-secretase-APPC complex, giving extended and more precise cleavage. (e) In contrast, fAD mutations favor the looser state and reduce the stability of the enzyme-substrate complex, leading to more diverse and longer peptides.

The role of conformational stability and hydrophobic membrane packing implied by computational correlation studies of the clinical data was shown experimentally by Szaruga et al., which convincingly showed that the shift towards longer peptides was consistent with a loss of enzyme-substrate complex stability, the structural reality probably underlying the kinetic model proposed by Okochi et al. Photo affinity studies showed the conformational effects of substrate positioning directly relevant to this context, pointing towards the involvement of looser conformations in the membrane relevant to substrate processing. Specifically, the fAD PS1 mutations alter the substrate interaction with the APP-C cleavage site of the enzyme, probably because mutations change the active site conformation. Such interaction changes are now known to be a general feature of fAD PS1 mutations, independent of the product line of Aβ generation ending with Aβ or
Aβ_{43}, and of PS1 autophagy. Crucially, the computer simulations performed by us have been well reproduced by excellent groups in this field. We note that Chávez-Gutiérrez and Szaruga in their recent excellent review refer to the stability effect as the metastable model, which fairly presents the concept that γ-secretase exists as several delicate conformation-sensitive states that can be perturbed by mutation or drug binding.

The FIST model also explains in a simple way why the amount of long and short Aβ depends on membrane thickness (which favors the more compact precise cleavage) and protein-conformation-dependent drug binding. In an important study, Winkler et al. showed that membrane thickness directly reduces the production of longer Aβ_{42/43} by pathogenic mutations. According to the FIST model, fAD PS1 mutations impair the stability and hydrophobic packing of the enzyme in the membrane, i.e. fAD mutations shift the two-state equilibrium towards the open state (looser squeezing by the “fist”), thus producing less stable enzyme-substrate complexes, less activity, earlier release, imprecise cleavage, and relatively more of the longer Aβ. In the first 3D structure and dynamic study of PS2-γ-secretase, a similar presence of a more compact and a more open state was observed in terms of the space between the catalytic aspartates, implying that PS2 and PS1 follow the same mechanism, at least qualitatively.

Since the experimental structures were identified at ~85 K without a membrane environment, it remains an open question whether some of the conformational details of the cryo-EM structures are not physiologically precise. Accordingly, the experimental 6IYC system (γ-secretase-APP-C_{83}-complex) has been simulated at physiological temperature (hot state; 300 K), liquid nitrogen temperature (cold state; 85 K) and in a rapidly cooled state. The 6IYC structural features resembled to the simulated cooled state. Both 6IYC and cooled state differed considerably from the hot state. Cryo-contraction of γ-secretase was quantified as a reduction in both the radius of gyration (R_g) and solvent accessible surface area (SASA) of the protein. The contraction was larger in membrane than in water. R_g and SASA in the cooled state structure were intermediate between the hot and cold states and closely resembled the experimental 6IYC structure, i.e. the real protein is likely to be more flexible and slightly larger than the cryostructure. The conclusions were consistent with earlier reported temperature-variable X-ray diffraction studies for ribonuclease and myoglobin and show that thermal dynamics are essential for structure-function relations of the enzyme, and thus MD
simulations are a necessary supplement to the cryo data, although these provide the necessary structural basis for these high-temperature dynamics.

5. THERAPEUTIC IMPLICATIONS OF THE FIST MODEL: NOTCH

Within the classical amyloid paradigm, many γ-secretase inhibitors were developed, but none succeeded in reaching the market.\textsuperscript{8,126-128} One possible reason is that γ-secretase processes more than hundred different substrates\textsuperscript{129} including also Notch, whose cleavage is important to many cell processes.\textsuperscript{130-132} Notch trimming resembles that of APP-C\textsubscript{99}\textsuperscript{133} producing the Notch intracellular domain (NICD) that also has a significant role in memory and learning.\textsuperscript{134,135} Understanding the role of γ-secretase in disease thus implies understanding this wider range of substrates, and the desire to target γ-secretase while sparing its effect on Notch could make these causal drugs more clinically efficient.\textsuperscript{136-138}

In terms of "Notch-sparing", it is imperative to study Notch in comparison with APP-C\textsubscript{99} in complex with γ-secretase. The experimental γ-secretase structures complexed with shorter APP-C\textsubscript{83}, an analogue of APP-C\textsubscript{99}, and Notch-100 have been derived by cryo-electron microscopy in 2019\textsuperscript{47,104} and provided the structural basis of the two modes of substrate binding.

MD simulations have been undertaken to understand the dynamics of the two substrates in a comparative context. These simulations were based on the experimental cryo-EM structures of wild-type γ-secretase complexed with APP-C\textsubscript{83} and Notch-100 but were then embedded in realistic membranes and simulated at physiological temperature.\textsuperscript{81}

One of the notable observations in such simulations was the repeated occurrence of β-strands in several solvent-exposed regions of presenilin,\textsuperscript{81} in agreement with other studies\textsuperscript{83,88} as also implied from the recent cryo-electron microscopy structures.\textsuperscript{47,104} However, this β-strand signal is weaker at physiological temperature than at cryo-temperature, consistently observed in two different studies.\textsuperscript{81,87} It remains unclear to what extent this strand motif plays a role in substrate binding and processing at physiological temperature where its stability remains to be established.

Another outcome of these molecular simulations was the finding that The RKRR motif (1758-1761) of Notch contributed persistently to Notch binding by acting as a highly charged (+4) and fixated membrane-anchor.\textsuperscript{81} Importantly, the Notch-bound complex persisted more in the closed conformational state with shorter distance between the catalytic aspartates. In contrast, the
corresponding less (+3) charged tri-lysine anchor (724-726) contributed less persistently to APP-C83 binding, mainly by interacting with E280 of PS1. This observation could perhaps explain the differential Notch and APP-C99 trimming by γ-secretase, with more imprecise trimming of APP-C99 in a more open state leading to more varied product formation.

6. MODULATING γ-SECRETASE FUNCTION

There are several possible reasons for the failure of direct inhibition of γ-secretase: First, the amyloid hypothesis may be incorrect. Second, Aβ peptides may be involved in AD but their production should not be inhibited because of crucial natural functions of this and other cleavage products in the brain. Third, the amyloid hypothesis may be valid but the inhibition of γ-secretase prevents the processing of other substrates such as Notch which then causes side effects before any positive effect is seen. Fourth, C-terminal fragments accumulating in the membrane could be toxic, and finally, the inhibitors may actually only be pseudo-inhibitors that still enable accumulation of long Aβ peptides. The cognitive decline could thus be caused by the non-selective inhibition of γ-secretase. Staying with the paradigm, many researchers have pursued the idea of "Notch-sparing" GSMs.

A prominent class of GSMs, phenylpiperidine-type modulators, bind towards the C-terminus of TM1 of PS1, which is the extracellular/luminal side that induces the conformational changes in the cytoplasmic side of PS1. Petit et al. reported that the extracellular interface between γ-secretase (NCT, PS) and substrate (APP) constitute the targets for GSMs. Very recently, Shi and co-workers elucidated a cryo-EM structure bound to inhibitors and a modulator showing their differential binding, with the modulator binding on the extracellular side of γ-secretase. Computer simulations indicate that pyridopyrazine-1,6-dione binds to a site between PS1-TM2, PS1-TM5 and the substrate. The experimental activities are primarily explained by the hydrophobicity, molecular size and polarizability, which contribute to the stabilization of the more compact semi-open PS1 state, thus favoring more precise and complete substrate trimming.

The molecular mechanisms of GSMs have not been well understood, which limits their rational design. However, the FIST model is not only a model of how fAD mutations work but also provides a plausible mechanism for the function of GSMs, the perhaps most promising current treatment strategy within the amyloid hypothesis paradigm. GSMs are intended to selectively...
reduce formation of longer pathogenic Aβ$_{42}$, while ideally sparing the cleavage of Notch.$^{147,152-154}$ Computational quantitative structure-activity relationship (QSAR) studies suggest that the binding affinity of the overall ternary complex enhances residence time of APP-C$_{99}$, thus ideally working oppositely to fAD mutations.$^{82}$

The GSMs are structurally diverse and have been categorized in three groups.$^{10,155-157}$ The first generation includes derivatives of nonsteroidal anti-inflammatory drugs (NSAID), which tend to reduce Aβ$_{42}$ while favoring the generation of shorter Aβ isoforms, such as Aβ$_{38}$. They function via allosteric modulation of the interaction between APP-C$_{99}$ and presenilin. Second-generation GSMs generally exhibit better efficacy and bioavailability. This class comprises acidic compounds (example, GSM-1) and non-acidic bridged aromatics (example, from Easai company), whose potency vary significantly.$^{156,157}$

QSAR is an important technique to understand the drug mechanisms, but relatively a few studies have used them to understand GSMs$^{158,159}$ probably due to the complex binding mechanism with several potential allosteric binding sites.$^{112}$ QSAR studies were performed on a compiled dataset of structurally diverse 60 GSMs using chemical and structural descriptors against the experimental EC$_{50}$ values (Figure 3c).$^{112}$ A chemically meaningful model described most of the potency with only 4 descriptors: The number of hydrogen-bond acceptor sites, the topology of the drug, the dehydration energy, and the binding energy to γ-secretase.$^{112}$ The model indicates a compromise between the binding energy to γ-secretase and the hydrogen-bond network and strong, non-specific binding of GSMs to several sites (Figure 3c, 3d). Potency is achieved notably by larger molecules with many hydrogen-bond acceptor sites, small dehydration penalty, and a favourable binding energy to γ-secretase. In the context of the FIST model, these compounds contribute their binding affinity to stabilize the ternary complex with APP-C$_{99}$ in the compact state that favors extended cleavage to shorter Aβ peptides.$^{112}$

The GSMs favors tight APP-C$_{99}$ binding thereby increasing retention time to form shorter Aβ peptides (Figure 3d), while the presenilin mutations favor the open state (Figure 3e) and decrease the retention time of the substrate to favour the production of longer Aβ homologs. The tight “grabbing” of the substrate by PS1, which acts as “fist”, was done mainly using TM2, TM3, TM6 and TM9 of PS1 which form the “fingers” of the “fist”. The fAD mutations in PS1 reduce the hydrophobicity, compactness and stability of the γ-secretase-substrate complex (Figure 3e), thus featuring the open
conformation,\textsuperscript{14,60,116} which enable an understanding of the experimental data,\textsuperscript{48} i.e. lower activity or higher A\textsubscript{42}/A\textsubscript{40} ratios, for most variants.\textsuperscript{48,49,116}

The NSAID derivative carboxylic acid modulators and non-acid modulators bind to presenilin\textsuperscript{146} with differential preferences for sites. They primarily target PS1-NTF (imidazoles and acids),\textsuperscript{147,160–163} however, a few preferably bind to PEN2.\textsuperscript{146,164} The N-terminal part of PEN2 interacts with TM4 of PS1\textsuperscript{165} and may modulate presenilin conformation and A\textsubscript{42} production.\textsuperscript{74,75,166} Collectively, these studies indicate that multiple modulator sites are present in \( \gamma \)-secretase\textsuperscript{163}, and that a specific class of compounds prefers a particular site. The main hotspot sites appear to be PS1-NTF and the interfaces between PS1-TM4 and PEN2, and PS1/nicastrin and APP-C\textsubscript{99}.\textsuperscript{116} Some studies indicate that the GSMs target TMs.\textsuperscript{146,147,167}

![Figure 4. The FIST mechanism in relation to the amyloid hypothesis.](image)

The semi-open protein conformation favours the substrate APP-C\textsubscript{99} processing. The binding of \( \gamma \)-secretase modulators (M) stabilize the protein-substrate complex, and thus, increase the retention time of the substrate and cause more trimming to form smaller A\beta peptides. The pathogenic fAD mutations reduce the hydrophobic packing and stability of the protein thereby favouring the open conformation of the protein and thus, impairing substrate retention and cleavage to form longer A\beta peptides.
7. CONCLUSIONS AND PERSPECTIVES

Alzheimer and dementia kill more than a million people every year, but no causal efficient treatments exist, showing the need to identify the molecular origins of disease. The strongest causal relationship is to γ-secretase and how it produces Aβ. Mutations in presenilin, the catalytic subunit of γ-secretase, produce severe early-onset familial AD (fAD). The last 3-4 years have seen major steps forward towards understanding fAD and has showcased the importance of computational science in integrating emerging cryo-electron microscopy structures, biochemical assays of Aβ formation by mutants, and clinical data on age of onset of variant carriers, via diverse computational modeling (molecular dynamics, QSAR, chemo- and bioinformatics).

The findings discussed here form the basis of the FIST (Fit-induce-stay-trim) model of γ-secretase (Figure 4)\(^{60,88,112}\), a model that holds that two conformations of the substrate binding site, a loose and a compact, controlled by membrane hydrophobic packing stability and defined by the distance between the catalytic aspartates, explains the pathogenicity of fAD mutations and the function of GSMs, the perhaps most promising current AD therapy, in addition to oligomer-selective antibodies. The semi-open conformation maximizes fitting (binding affinity) of the substrate leading to longer retention time and extended cleavage to shorter Aβ peptides, and the open state (looser holding by the fist) reduces staying time and trimming and leads to higher Aβ\(_{42}/Aβ_{40}\) ratios.\(^{112}\)

Despite the findings presented here, we note that the molecular etiology of fAD remains debated.\(^{5,7,64,168}\) We have discussed one plausible molecular mechanism of γ-secretase, which can explain the currently known genetic, biochemical, and clinical data of fAD, including the significance of the Aβ\(_{42}/Aβ_{40}\) ratio, and a plausible mode of function of PS1 mutations. As discussed, the semi-open state exists in equilibrium with a state with more space in the catalytic pocket that gives rise to imprecise cleavage along both cleavage pathways, and less complete trimming, leading to a larger fraction of longer peptides being formed. The FIST model suggests that fAD mutations favor the loose state and destabilizes the enzyme-substrate complex by loss of hydrophobic packing.

The FIST model also points to treatment regimes that balance the processing by γ-secretase rather than destroys its function, notably by stabilizing the semi-open “innocent” conformation (tight grabbing by fist) and contributing stability to a ternary modulator-enzyme-substrate complex in a way that reconstitutes the semi-open innocent conformation state of the enzyme, which mainly produces Aβ\(_{40}\) and other cleavage products with plausibly important functions in the brain\(^5\) (both Aβ\(_{40}\) and Aβ\(_{42}\)
are reported to be neuroprotective at low concentrations.\textsuperscript{169,170} The modulator-favoured more compact conformation state increases the retention time of the substrate for maximum cleavage to shorter Aβ homologs.\textsuperscript{112,116} The model thus serves as a \textit{modus operandi} for future development of therapeutics against AD with a molecular mechanistic underpinning, but first and foremost illustrates the unique importance that multiscale computer modelling plays in integrating the diverse complex data of modern biology and medicine into coherent, testable causal disease mechanisms.

\textbf{Conflicts of interest}

The authors declare that they have no conflicts of interest associated with this work.

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