The Same Defendant

C99 in Alzheimer's disease: one uncut fragment, three compartments, three lesions

Benjamin Aaron Gustafsson

AdultCognitiveDisease.com

November 2026

Cutting the amyloid precursor protein takes two operations, and the field has spent thirty years studying what comes off the end of the line. Three laboratories, working in different compartments for different reasons, have concluded that the damage is done by the half-finished piece waiting between the cuts — and a fourth, with no stake in the question, agrees.

Contents

How to read this paper
1. The Fragment Nobody Was Watching
    1.1 Two cuts
    1.2 Where the first cut happens
    1.3 The reversal
    1.4 Why this paper exists
2. What the Fragment Does for a Living
3. First Crime Scene: The Contact Site
    3.1 Where this happens
    3.2 The finding
    3.3 The false alarm
    3.4 Two lanes to one disease
4. What the Product Is For, and What Steers the Cut
    4.1 The peptide looks like a dial
    4.2 The second cut is not one cut
    4.3 Two things set the grip, and they are not rivals
    4.4 The loop
5. Second Crime Scene: The Acid Pump
    4.1 What the pump is for
    4.2 The finding
    4.3 The detail that joins this section to the last
6. Third Crime Scene: The Neuron, With the Peptide Removed
7. The Witness With No Stake
8. What the Convergence Is Worth
9. The Valence Question, Left Open
10. Where the Fragment Sits in Our Model
11. The One Measurement That Would Discriminate
12. Graded Ledger
13. What Would Change This Account
Companion papers in this corpus
References

Abstract

When the amyloid precursor protein is cut for the first time, it leaves behind a ninety-nine-residue stub embedded in the membrane. The textbook calls it C99 and treats it as a way-station: an enzyme comes along, makes the second cut, amyloid-β is released, and the disease story begins there. It is also usually said to be made at the cell's outer surface. It is not: the enzyme that makes it needs acid, and the healthy neuron keeps enzyme and substrate deliberately apart until endocytosis brings them together inside the cell. The fragment is born in the endosome.

Three research programmes, working in different compartments with different methods and no shared agenda, have independently concluded that the story begins earlier — with the stub itself. This paper sets their three findings side by side, which has not been done before in this corpus or, so far as we can tell, anywhere else.

At the **contact site** where the cell's internal membrane system touches its mitochondria, Estela Area-Gómez's laboratory finds that C99 behaves as a cholesterol sensor. Its accumulation is read by the cell as a shortage of cholesterol — a false alarm it cannot detect — and the cell answers by hauling cholesterol inwards and stashing it, in a cycle that never switches off because the signal that started it is the pile itself.

At the **lysosome**, Ralph Nixon's laboratory finds that the same fragment, phosphorylated on a single tyrosine, binds into a pocket on the acid pump and physically prevents the rest of the pump from assembling. Lower that phosphorylation and acidification recovers; lower it below normal and the pump runs better than baseline, which suggests the fragment is tuning that pump in health as well as breaking it in disease.

Inside the **neuron itself**, Frédéric Checler's laboratory finds that C99, expressed without any amyloid-β at all, produces the early intraneuronal lesions and the autophagic pathology that had been attributed to the peptide.

A fourth group with no stake in any of this built a large panel of genetically matched human neurons carrying familial mutations and asked which molecule accounted for their shared defects. The answer was the fragment, not the peptide.

Two of those programmes hold gold Oskar Fischer Prizes. The paper argues that this convergence is worth more than any one of the three findings, and considerably less than a proof of causation — and it is written so that nothing in it depends on the most contested claim the three share, which is that the fragment matters more than the peptide it becomes.

How to read this paper

This is a standalone paper about one molecule. It belongs to no series. It assumes nothing except that the reader knows Alzheimer's disease involves a protein that gets cut.

It also has an unusual purpose, which should be declared. This corpus has used C99 extensively — it appears twenty-seven times in our evaluation of Nixon's programme alone — while naming none of the people whose central claim it is. The material was absorbed and the architecture was lost. This paper is partly a repair of that, which is why attribution is stated more explicitly throughout than house style would normally require.

Claims are graded where made. **Established** means replicated in human material by more than one group. **Probable** means good evidence with a gap named. **Inference** means a conclusion drawn here from evidence gathered for another purpose. **Speculative** means coherent and untested.

One naming convention. The fragment is called **C99** by the laboratories that work on lipids and **βCTF** — β-carboxy-terminal fragment — by those that work on membrane traffic. They are the same object. This paper uses C99 throughout and notes the other name where a cited study uses it, because the two vocabularies are part of why these literatures took so long to meet.

1. The Fragment Nobody Was Watching

1.1 Two cuts

The amyloid precursor protein sits in the membrane with a long piece outside the cell and a short tail inside. Making amyloid-β takes two cuts. The first, by an enzyme called β-secretase, lops off the outside piece and leaves a ninety-nine-residue stub still embedded in the membrane: **C99**. The second, by γ-secretase, cuts within the membrane and releases amyloid-β.

In the standard account the stub is of no independent interest. It exists briefly between two enzymes and the pathology begins when the second cut delivers its product.

1.2 Where the first cut happens

It is usually said that the first cut is made at the surface of the neuron. That is the older picture, and the biochemistry is against it.

The constraint is acid. β-secretase — BACE1 — works best at a pH near 4.5. The outer face of the plasma membrane faces the outside world at pH 7.4. An enzyme with an acidic optimum is in the wrong place there. The compartments that are actually acidic are the endosome and the lysosome.

The neuron keeps them apart on purpose. The precursor protein and BACE1 are both abundant in brain, so the real question is not how they meet but why they are not meeting constantly. Working in cultured hippocampal neurons, Das and colleagues found that after synthesis the two are largely segregated: BACE1 is sorted into acidic recycling endosomes, while the precursor protein travels in a separate set of Golgi-derived vesicles. They are routed on different lines. On activity induction — a known trigger of the amyloidogenic route — the precursor protein is drawn into the BACE1-positive endosomes by a clathrin-dependent mechanism, which is to say by endocytosis. The same partitioning is seen in control brain and the same convergence in Alzheimer brain (Das et al., 2013).

The conclusion the authors draw is the one this paper needs: **the convergence of enzyme and substrate is the rate-limiting, obligatory event**, and it is a trafficking event rather than a surface event. **Established.**

So C99 is not made at the surface and delivered inward. **It is born in the endosome**, because that is where the cell has arranged for its enzyme to be, and the arrangement is normally one of separation.

A third address, and an unresolved tension. Area-Gómez's programme places the processing line somewhere else again — at the ER–mitochondrial contact of Section 3. Working in Nice, Del Prete and colleagues reported that the precursor protein and its fragments are present at those contacts and that **both** β- and γ-secretase are present there and carry processing activity, in cells overexpressing the precursor and in the brains of transgenic mice (Del Prete et al., 2017). That is a real result and it is the address her account requires.

It also sits awkwardly with the paragraph above, and this paper does not resolve it. The contact is an ER domain at roughly neutral pH, and BACE1's optimum is acidic. The programme's answer would presumably be that the contact is a cholesterol-rich, lipid-ordered domain in which the raft-preferring secretases concentrate and their activity is modulated, so that residence substitutes for acidity. Whether that is sufficient, or whether the contact-site pool is a minor one beside the endosomal pool, is not established either way, and the supporting work is in overexpressing systems. **Recorded as open.** It matters for Section 3, because if the fragment is made in the endosome then getting it to the contact site is a trafficking step her account needs and does not yet specify.

1.3 The reversal

The claim this paper is about inverts that. On this reading the fragment is the agent, and the second cut is not the beginning of a pathology but the **termination of a signal** — the step that switches the fragment off. The pathology is what happens while the fragment waits.

Checler's group put the inversion in a review title, as a question: is γ-secretase a beneficial inactivating enzyme for a toxic fragment? (Checler et al., 2021). If the answer is yes, then the familial mutations in the presenilins — the catalytic core of γ-secretase — are loss-of-function mutations, and much of the field's architecture has to be re-read.

An everyday version. A workshop cuts a part in two operations. Everyone has spent thirty years studying the finished part that comes off the end of the line, because that is what piles up in the yard. The claim is that the damage is done by the half-finished piece sitting on the bench between operations, and that the second cut is not the moment the dangerous thing is created but the moment it is made safe.

1.4 Why this paper exists

Taken from any single laboratory, this would be one more contrarian reading in a field with many. What makes it worth a paper is that **three laboratories, working on different compartments, with different techniques, for different reasons, arrived at the same defendant** — and that a fourth group with no interest in the question tested it and agreed.

Sections 3 and 5 to 7 take them one at a time, and Section 4 asks what the product of the second cut is for. Section 8 states carefully what the convergence is worth, which is less than it first appears.

2. What the Fragment Does for a Living

Before the three crime scenes, the motive. C99 is not an inert stub; it has a measurable physical property, and that property was established by people with no stake in the disease argument.

Charles Sanders's group studied the fragment by nuclear magnetic resonance — a technique that reports the shape and flexibility of a molecule in solution. They found that its membrane-spanning segment is unusually flexible and that it **binds cholesterol directly**, at a defined surface just upstream of the γ-secretase cut site (Beel et al., 2008; Beel et al., 2010; Barrett et al., 2012). They had raised the possibility that the precursor protein functions as a cholesterol sensor years before anyone attached it to this disease.

Established, by structural methods, independently of the disease literature.

One further result from that group matters more than it is usually given credit for. Cholesterol binding is **in competition with the fragment's tendency to stick to itself** (Song et al., 2013). At high concentration C99 prefers its own company to the sterol's.

That detail deserves a moment. It means accumulation does not merely produce more of the same activity — it changes what the molecule does. A fragment present at low concentration senses cholesterol; the same fragment

at high concentration increasingly dimerises instead. A quantity becomes a different quality, which is exactly the kind of non-linearity a disease of slow accumulation would need and which a simple dose-response picture misses. **Established** in vitro; its consequences in a neuron are taken up by recent work on dimerisation and proteostasis (Badot et al., 2026) and are not settled.

3. First Crime Scene: The Contact Site

3.1 Where this happens

Cells are not bags. Their internal compartments touch each other at defined places, and at those contacts they exchange material. One such contact is between the endoplasmic reticulum — the membrane system where lipids are made and quality-controlled — and the mitochondria. The contact zone has a name, **mitochondria-associated ER membranes**, usually shortened to MAM, and it is where a cell does much of its lipid and calcium housekeeping.

It is also, as Area-Gómez's group showed early, where the presenilins are enriched (Area-Gómez et al., 2009). The enzyme that makes the second cut lives at the contact site, which is why the fragment's fate is decided there.

3.2 The finding

In Alzheimer's disease, the function of these contacts is **upregulated** — not lost (Area-Gómez et al., 2012). That direction matters, and it is the same direction the corpus's return-leg work keeps meeting: a machine running harder, not a machine stopped.

The mechanism proposed for it is the programme's central claim. C99 delivered to the ER acts as a **lipid-sensing peptide**. Its presence organises cholesterol-rich domains, and in cellular models of the disease raised levels of uncleaved C99 upregulate the formation of those domains by inducing the internalisation of cholesterol from outside the cell and its trafficking from the cell surface inwards to the ER (Montesinos et al., 2020; reviewed by Agrawal et al., 2020). Increased C99 at these contacts produces mitochondrial dysfunction (Pera et al., 2017).

Probable. The cellular work is strong and from one group; the human-tissue arm is thinner.

3.3 The false alarm

The interpretation is what makes it a disease mechanism rather than a curiosity, and it is worth stating in plain words.

The cell has no way to ask *why* there is a lot of C99. It only reads the signal, and the signal means *cholesterol needs handling*. So an accumulation caused by a failure of cutting is read as a demand for cholesterol, and the cell answers by importing cholesterol and esterifying it for storage. That answer does not reduce the C99, because C99 was never the shortage. The alarm therefore never clears, and the cell pays the cost of answering it indefinitely.

This is the same shape the corpus has found elsewhere: a homeostatic correction left switched on because the thing it corrects for is not what triggered it. It is recorded here as **Inference** — the mechanism is the programme's, the identification of the shape is ours.

3.4 Two lanes to one disease

The claim generates a unification. Familial and sporadic Alzheimer's become the same disease reached by two routes: in the familial form, mutations raise C99 directly and the C99 drives cholesterol to the ER; in the sporadic form, cholesterol delivery to the ER rises first, for any of several reasons, and the raised cholesterol raises C99. Either way the contacts are upregulated and the downstream is common.

It is a stronger claim than it looks, because it predicts two things that can be checked. Sporadic tissue should show elevated C99 **despite normal** *APP* **and** *PSEN* **genes** — which is reported. And the sporadic risk architecture should be enriched for genes that move cholesterol — which, with *APOE* at its head, it is. The programme supplies the mechanism for that head: ApoE4 upregulates the activity of these contacts (Tambini et al., 2016).

Probable, and it is the most testable thing the programme says.

4. What the Product Is For, and What Steers the Cut

If the fragment is the agent, an obvious question follows: what is the *product* for? A cell that has evolved a two-step machine to make amyloid-β presumably makes it for a reason, and the reason bears on how the second cut is controlled.

4.1 The peptide looks like a dial

Amyloid-β is not only made in disease. It is made constantly, by healthy neurons, and its production is coupled to how hard they are working.

Malinow's group showed that neuronal activity modulates the formation and secretion of the peptide, and that the peptide in turn **depresses** excitatory transmission — onto the neurons producing it and onto neighbours that are not. The depression requires NMDA-receptor activity and is reversed by blocking neuronal firing. Their proposal, stated in the paper, is that activity-dependent production normally participates in a **negative feedback that keeps neuronal hyperactivity in check** (Kamenetz et al., 2003). **Established** in that system; the work was done in slices overexpressing the precursor protein, which is the caveat.

Set beside the other direction — that at picomolar concentrations amyloid-β42 markedly increases long-term potentiation and enhances memory (Puzzo et al., 2008) — the shape is biphasic. A little improves; a lot suppresses. That is the signature of a regulator rather than of a waste product, and a framework that treats the molecule as having only its pathological concentration is not describing the biology.

So the first answer to what steers production is simply **synaptic activity**. Busy neuron, more peptide, quieter neuron.

4.2 The second cut is not one cut

The mechanical answer is more interesting, and it rests on a fact about γ-secretase that is often lost in summary.

The enzyme does not make a single cut and release a defined product. It makes a first incision and then walks along what remains, trimming in roughly three-residue steps and releasing a **distribution** of peptide lengths. Amyloid-β40, amyloid-β42 and the shorter species are all products of the same enzyme stopping at different points along the same substrate.

What is being steered, therefore, is **processivity** — how long the enzyme keeps hold before letting go. Hold on through more steps and the products are shorter and less aggregation-prone; release early and the longer, stickier species are what emerge. The field's most-cited molecular index of this disease, the ratio of amyloid-β42 to amyloid-β40, is mechanically a measurement of grip.

4.3 Two things set the grip, and they are not rivals

The enzyme–substrate complex's own stability. Chávez-Gutiérrez and De Strooper's group showed that as the substrate shortens, each successive enzyme–substrate complex becomes progressively less stable — the grip is already weakening at every step. Alzheimer's-causing mutations in presenilin or in the precursor protein destabilise those labile complexes further, so the enzyme lets go early and longer peptides are released (Szaruga et al., 2017). **Established**, and it is the dominant explanation in the field.

The detail that bears on the present question is what they did next. Destabilising **wild-type** complexes — by temperature, by compounds, or by detergent — produced the same shift toward longer peptides, while stabilisers increased processivity. The authors draw the inference explicitly: environmental factors may raise risk. The grip is physically tunable by its surroundings, in an enzyme carrying no mutation at all.

The thickness of the membrane the cut happens in. This is Area-Gómez's most audacious proposal and it follows directly from Section 3. Because the enzyme cuts *within* the membrane, the geometry of engagement depends on how thick that membrane is. In a raft of normal thickness the substrate's helix matches the bilayer and is cut predominantly to the shorter peptide. Thin the raft and the helix must tilt to maintain hydrophobic matching; the tilt changes its alignment with presenilin-1; and the cut falls in a different register, favouring the longer species. On that reading the 42:40 ratio is **a surrogate for membrane thickness — a ruler rather than a poison**.

The programme names the enzymes that would do the thinning, both resident at the contact: ACAT1, and the long-chain acyl-CoA synthetase ACSL4, which preferentially activates polyunsaturated chains. Kinked chains are effectively shorter than straight ones, so loading them into the domain's phospholipids thins it.

Our verdict on the pair, which the corpus has stated before and repeats here. These are not competing mechanisms. Processivity is the proximate description; thickness is a proposed *modulator* of it, and the modulation appears to be real. But the programme's summaries present thickness as the explanation rather than as a contribution to one, and the quantitative question — how much of the ratio shift observed in human disease is attributable to membrane geometry — has not been answered by anyone. Until it is, the thickness account is a hypothesis with a named mechanism rather than a finding. **Probable** as a contribution; **not established** as the explanation.

4.4 The loop

Put Section 3 and this section together and something closes, which is the reason this section exists.

The fragment senses cholesterol. Its accumulation drives cholesterol inward and remodels the lipid composition of the contact. And that lipid composition sets the geometry of the cut **that destroys the fragment**.

So the substrate governs the membrane that governs the enzyme that disposes of the substrate. The sensor sits on its own thermostat.

Read through that loop, the question this section opened with answers itself in a specific direction. Amyloid-β is not steered into existence as a by-product of a failure. It is the **output of a homeostat with two inputs** — how active the neuron is, and what state its membranes are in — and the species released reports on both. On this reading the peptide is a readout, and the disease is what happens when a readout is driven by a signal that cannot clear.

Inference, ours, and untested. Nobody has closed that loop experimentally in a neuron: the steps are individually supported and have never been demonstrated as a cycle in one system. It is stated because it is cheap to test and because it sharpens what to look for — a measurement of membrane thickness, fragment load and peptide-length distribution in the same cells, across a manipulation of any one of the three.

What it does not do is settle the valence question of Section 9. A molecule can be a regulator *and* be what does the damage when the regulator is held on, and nothing in this section decides between those.

5. Second Crime Scene: The Acid Pump

4.1 What the pump is for

A lysosome is the cell's disposal unit, and it works by being acidic. It holds a pH near 4.5, maintained by a molecular pump in its membrane that drives protons inward, and its digestive enzymes do not function without that acidity. If the pump fails, nothing downstream gets digested, whatever else is working.

Our evaluation of Nixon's programme describes that failure at length. What it did not say — and what this section repairs — is what *causes* it.

4.2 The finding

Nixon's laboratory identified the agent, and the mechanism is unusually concrete for this field.

The β-cleaved fragment — C99, written βCTF in this literature — when phosphorylated on a particular tyrosine, binds **selectively into a pocket on the V0a1 subunit** of the pump's membrane portion, and by occupying that pocket it **competitively blocks the pump's other subcomplex from docking**. The pump cannot assemble, so it cannot pump, so the compartment cannot acidify (Im et al., 2023).

Three features of that result make it stronger than a correlation. It is **reversible**: lowering the phosphorylation restores pump activity and lysosomal function both in human Down syndrome fibroblasts and in the brains of Down syndrome model mice. It is **bidirectional**: lowering phosphorylation *below* normal constitutive levels raises pump assembly and activity above baseline, which implies the fragment modulates this pump tonically, in health, and not only as a disease lesion. And it **generalises**: elevated phosphorylation disrupts pump function in two mouse models of Alzheimer's disease as well.

Established in human fibroblasts and mouse brain. Not yet demonstrated in human brain tissue.

4.3 The detail that joins this section to the last

The sequence of C99 that binds the pump is its **YENPTY motif**. That motif's ordinary job, in a healthy cell, is to act as an address label: it is the tag that tells the cell's machinery to pull this protein in from the surface.

Section 1.2 says what it pulls it *into* — the acidic compartment where BACE1 is waiting. So this one short sequence does both jobs in the story. It delivers the precursor protein to the enzyme that makes the fragment, and then, phosphorylated, the fragment uses the same sequence to disable the acid pump further down the same pathway. The instruction that creates C99 and the lesion C99 inflicts are written in the same residues.

The entry instruction and the disposal lesion are one piece of sequence. **Inference**, ours, for the joining of the two findings; each finding is established separately.

That is the tightest available weld between the corpus's lipid material and its lysosomal spine, and our own integration audit flagged it as missing before this paper was written. It also has consequences upstream: the fragment binds APPL1, an effector of the small protein Rab5 that governs the early endosome, and recruits it to the endosomal membrane (Kim et al., 2016); over-activating Rab5 by itself reproduces prodromal and neurodegenerative features including cholinergic degeneration (Pensalfini et al., 2020). And downstream: faulty acidification produces an autophagic build-up inside neurons that ends in the cell dying in place (Lee et al., 2022).

Asked directly in November 2026 what tips the endosome off balance in the common form of the disease, Nixon answered that the C-terminal fragment, cholesterol and *APOE* ε4 are all tributaries to one river — Rab5 activation. Recorded as his framing, given in correspondence, **not yet published in that form**. It is notable here because two of his three tributaries are the other programme's two variables.

6. Third Crime Scene: The Neuron, With the Peptide Removed

The obvious objection to everything above is that C99 and amyloid-β travel together. Wherever there is more of one there is usually more of the other, so an effect attributed to the fragment may belong to the peptide.

Checler's group in Nice did the experiment that answers it. Working in transgenic and viral systems in which C99 could be expressed and studied **without** amyloid-β, they showed that the fragment, not the peptide, is the key contributor to the early intraneuronal lesions of a widely used mouse model (Lauritzen et al., 2012), and that intraneuronal aggregation of C99 produces lysosomal and autophagic pathology that is demonstrably amyloid-β-independent (Lauritzen et al., 2016).

Established in those models. The limitation is that they are models, and that expressing a fragment at non-physiological levels can produce effects a physiological level would not.

7. The Witness With No Stake

One more result deserves its own section, because its value lies entirely in who produced it.

Marc Tessier-Lavigne's group built a large panel of human induced-pluripotent-stem-cell neurons carrying familial *APP* and *PSEN1* mutations, all on a **common genetic background** — isogenic, so that differences between lines are attributable to the mutations rather than to donor variation. They then asked which molecular species accounted for the abnormalities the mutations shared.

The answer, stated in the paper's own title: the shared endosomal abnormalities are mediated by β-C-terminal fragments, **not** by amyloid-β (Kwart et al., 2019).

Human cells. Isogenic. A large panel. And a laboratory with no commitment to the lysosomal account, the lipid account, or the fragment. **Established**, and it is the single most persuasive external corroboration any of the three programmes has.

8. What the Convergence Is Worth

Here the paper has to be careful, because convergence is the kind of argument that flatters whoever makes it.

What it does establish. Three laboratories using different systems — contact-site biochemistry, lysosomal cell biology, amyloid-free expression models — each concluded that this fragment does damage. A fourth, indifferent, agreed. Independent arrival by different methods is a real argument against method artefact: it is unlikely that three different techniques each generate the same false positive about the same molecule. **The fragment is independently pathogenic at three addresses** is, in our reading, **Established**.

What it does not establish. Three groups agreeing that a molecule does damage is not a demonstration that it does the *most* damage, nor that it acts *first*, nor that amyloid-β does not also do damage by its own routes. Convergence speaks to reality; it is silent on primacy. A molecule can be genuinely harmful in three compartments and still be a secondary player in the disease.

And one thing to watch. The three programmes are not perfectly independent of each other. They read each other's papers, they cite each other, and they share a vocabulary and a prior disposition against the amyloid cascade. Methodological independence is strong here; intellectual independence is weaker than the word "convergence" suggests. **Inference**, ours, and a caution against our own argument.

9. The Valence Question, Left Open

This paper now states explicitly what it is not claiming, because the claim it declines is the one a reader will assume it is making.

All three programmes share a position beyond the one this paper argues: that the fragment matters **more** than the peptide, and in the strong form that amyloid-β is a bystander or even a response. That is the substrate reversal, and the corpus does not hold it.

Our reasons are not electrophysiological or lipidological; they are genetic and therapeutic. The dominantly inherited mutations sit in the amyloid pathway and cause the disease with near-complete penetrance. The protective *APP* A673T variant reduces amyloid-β production and protects against the disease. And removing amyloid slows decline, modestly but measurably. Against those, the reversal offers a reinterpretation of each — the mutations act by disturbing a developmental process, the trials are confounded — and reinterpretation is what a framework does when the primary data run the other way.

But none of this paper's argument requires that question to be settled. A fragment can jam an acid pump whether or not it is the disease's prime mover. It can produce a false cholesterol alarm whether or not the peptide it becomes is also toxic. The three crime scenes are findings about what the molecule does; they are not votes on what causes Alzheimer's disease. We have written the paper so that every claim in Sections 3 to 7 survives either answer to the valence question, and readers who hold the opposite view from ours should be able to use it unchanged.

What the convergence does do is locate the fork precisely. **C99 is where the two readings diverge** — one side says the danger is made at the second cut, the other says it is ended there. That is a sharper statement of the disagreement than the field usually manages, and sharpening a disagreement is worth doing even when you cannot resolve it.

10. Where the Fragment Sits in Our Model

A companion paper, *The Long Way Back*, argues that the cell's sorting and recycling machinery is not a fourth step of the disease but an axis running beside all three — with a station in the brainstem neuron of Phase I, the disposal machinery of Phase II, and the synapse of Phase III.

C99 does the same thing one level further down. It is not a compartment or a machine; it is a molecule, and it is the molecule standing at that axis's junctions.

- **At the Phase I station**, it is upstream of the transport failure: the fragment recruits APPL1 to the early endosome and drives Rab5 activation, and Rab5 over-activation alone is sufficient to produce cholinergic degeneration.
- **At the Phase II station**, it is the thing jamming the acid pump, which is the step on which both the neuron's and the microglion's disposal depends.
- **At the contact site**, it runs a lipid cycle that no phase of our model currently owns — which is the honest answer to where Area-Gómez's work has been sitting, which is nowhere.

Inference, ours. No study has placed the fragment at all three stations in one system, and the three addresses have never been measured in the same cells.

11. The One Measurement That Would Discriminate

The programme's most actionable proposal is also its cheapest, and it is the right place to end.

The diagnostic quantity, on this reading, is not the amount of amyloid-β. It is a **ratio**: total amyloid-β to C99 — product to substrate — which reports

whether the cutting enzyme is keeping up with its workload. The proposal is to measure it in peripheral blood mononuclear cells, which is to say in white cells from an ordinary blood draw.

The reason it is not routine is a reagent problem the programme itself identified: many antibodies raised against amyloid-β **cannot distinguish it from the larger fragment that contains it**, because the fragment contains the sequence the antibody was raised against. That is a solvable assay problem, not a conceptual one.

Two things make this worth more than a footnote. It would be a **rate** read from a single sample, and our paper on measurement argues that rates are the quantity this field almost never obtains in a living person. And it is **discriminating**: if the substrate reversal is right the ratio should separate disease from ageing better than either quantity alone and should move earlier, and if it is wrong it should not.

We record it as an inventory item rather than as a recommendation. It has not been run at scale, and its behaviour across the staging series is unknown. **Inference**, ours, that it is the cheapest unbuilt read-out in the corpus; the proposal itself is the programme's, and was stated in advance of the data.

12. Graded Ledger

#	Claim	Grade	Resting on	What would overturn it
1	C99 binds cholesterol directly, at a site just upstream of the γ-secretase cut	Established	Beel 2008, 2010; Barrett 2012 (NMR, independent of disease work)	A structural study finding no specific binding
2	Cholesterol binding competes with C99 self-association, so accumulation changes its behaviour	Established in vitro	Song 2013	Demonstration that dimerisation does not displace sterol binding at physiological levels

#	Claim	Grade	Resting on	What would overturn it
3	The precursor protein and BACE1 are segregated in healthy neurons and converge on endocytosis; the first cut is made in the endosome, not at the surface	Established	Das 2013 (cultured neurons; control and Alzheimer brain)	A demonstration of substantial surface β-cleavage at neutral pH
4	Peptide length is set by γ-secretase processivity, and the grip is destabilised by temperature, compounds or detergent even in wild-type enzyme	Established	Szaruga 2017	A demonstration that peptide length is set independently of enzyme–substrate complex stability
5	Contact-site function is upregulated, not lost, in Alzheimer's disease	Probable	Area-Gómez 2012; Pera 2017 (one group)	An independent series finding contact-site function reduced
6	Raised C99 drives cholesterol inward and upregulates these contacts	Probable	Montesinos 2020 (cellular models, one group)	Failure to replicate the trafficking effect elsewhere
7	ApoE4 upregulates contact-site activity	Probable	Tambini 2016	Independent work showing no genotype effect
8	Phosphorylated C99 inhibits the lysosomal acid pump by blocking its assembly	Established (human fibroblasts, mouse brain)	Im 2023; reversible and bidirectional	Failure to replicate outside the originating laboratory; or no effect in human brain tissue
9	The pump-binding sequence is the protein's internalisation motif	Established	Im 2023	—
10	C99 without amyloid-β produces intraneuronal and autophagic pathology	Established (models)	Lauritzen 2012, 2016	Demonstration that the effect requires non-physiological expression

#	Claim	Grade	Resting on	What would overturn it
11	Shared endosomal abnormalities of familial mutations are mediated by the fragment, not the peptide	Established (human isogenic neurons)	Kwart 2019	Failure to replicate in an independent isogenic panel
12	The fragment is independently pathogenic at three addresses	**Inference (ours)**	Sections 3, 5–7 read together	A demonstration that two of the three effects share a single artefact or a common upstream cause
13	The three programmes are methodologically independent but not intellectually independent	**Inference (ours)**	Section 8	—
14	C99 is the molecule at the junctions of the endosomal axis	**Inference (ours, untested)**	Section 10	The three addresses measured in one system and found unrelated
15	The fragment, the membrane it remodels and the enzyme that destroys it form a closed loop	**Inference (ours, untested)**	Sections 3 and 4 read together; no step shown as a cycle in one system	Membrane thickness, fragment load and peptide-length distribution measured together and found uncoupled
16	Amyloid-β is pathogenic and the substrate reversal is not adopted	Probable; **contested by all three programmes cited here**	Dominant mutations; A673T; trial effects	A trial result or genetic finding that the reversal predicts and the cascade does not
17	The amyloid-β to C99 ratio is the cheapest unbuilt human read-out in the corpus	**Inference (ours)**	Section 11; the reagent problem is the programme's own diagnosis	A cheaper validated candidate; or the assay proving uninformative at scale

13. What Would Change This Account

The pump result replicated, or not, outside Nixon's laboratory. Claim 6 is the strongest single mechanism in the paper and it rests on one group. It is also the most falsifiable thing here, because the manipulation is specific and the read-out is quantitative.

The three addresses measured in one system. Nobody has asked whether the same fragment, in the same cells, is doing all three things, or whether three groups have each found a local effect of a molecule that is simply abundant wherever cells are sick. That experiment would settle claim 10 and it is not conceptually difficult.

A human-tissue demonstration of the pump lesion. Everything in Section 5 is fibroblasts and mice.

The ratio, measured. If the amyloid-β to C99 ratio in blood cells failed to separate disease from ageing, the programme's own most explicit prediction would have failed, and the substrate reversal would be in serious trouble — which is the strongest reason to want the assay built.

Companion papers in this corpus

The Uncleared Signal — Area-Gómez's programme assessed in full, with an audit of her 2020 predictions against the record since. *The Acid Test* — Nixon's endosomal–lysosomal–autophagy account assessed in full. *The Long Way Back* — the endosomal axis, and why it is not a fourth phase. *The Dial and the Decade* — what can and cannot be measured in a living person; Section 11 above is an entry in its Tier one. *The Other Half of the Cycle* — the register of recovery steps, to which clearance of this fragment has now been added.

References

Area-Gómez E, de Groof AJ, Boldogh I, Bird TD, Gibson GE, Koehler CM, Yu WH, Duff KE, Yaffe MP, Pon LA, Schon EA. Presenilins are enriched in endoplasmic reticulum membranes associated with mitochondria. *American Journal of Pathology*. 2009;175(5):1810-1816. PMID 19834068.

Area-Gómez E, del Carmen Lara Castillo M, Tambini MD, Guardia-Laguarta C, de Groof AJW, Madra M, Ikenouchi J, Umeda M, Bird TD, Sturley SL, Schon EA. Upregulated function of mitochondria-associated ER membranes in Alzheimer disease. *EMBO Journal*. 2012;31(21):4106-4123. PMID 22892566.

Agrawal RR, Montesinos J, Larrea D, Area-Gómez E, Pera M. The silence of the fats: a MAM's story about Alzheimer. *Neurobiology of Disease*. 2020;145:105062. PMID 32866617.

Badot C, Bini A, Duplan E, Checler F, Lauritzen I. Functional relationships linking C99/APP-βCTF dimerization, proteostasis disruption, and organelle dysfunction. *Cell Communication and Signaling*. 2026;24(1). PMID 42098771.

Barrett PJ, Song Y, Van Horn WD, Hustedt EJ, Schafer JM, Hadziselimovic A, Beel AJ, Sanders CR. The amyloid precursor protein has a flexible transmembrane domain and binds cholesterol. *Science*. 2012;336(6085):1168-1171. PMID 22654059.

Beel AJ, Mobley CK, Kim HJ, Tian F, Hadziselimovic A, Jap B, Prestegard JH, Sanders CR. Structural studies of the transmembrane C-terminal domain of the amyloid precursor protein (APP): does APP function as a cholesterol sensor? *Biochemistry*. 2008;47(36):9428-9446. PMID 18702528.

Beel AJ, Sakakura M, Barrett PJ, Sanders CR. Direct binding of cholesterol to the amyloid precursor protein: an important interaction in lipid-Alzheimer's disease relationships? *Biochimica et Biophysica Acta*. 2010;1801(8):975-982. PMID 20304095.

Checler F, Afram E, Pardossi-Piquard R, Lauritzen I. Is γ-secretase a beneficial inactivating enzyme of the toxic APP C-terminal fragment C99? *Journal of Biological Chemistry*. 2021;296:100489. PMID 33662398.

Das U, Scott DA, Ganguly A, Koo EH, Tang Y, Roy S. Activity-induced convergence of APP and BACE-1 in acidic microdomains via an endocytosis-dependent pathway. *Neuron*. 2013;79(3):447-460. PMID 23931995.

Del Prete D, Suski JM, Oulès B, Debayle D, Gay AS, Lacas-Gervais S, Bussiere R, Bauer C, Pinton P, Paterlini-Bréchot P, Wieckowski MR, Checler F, Chami M. Localization and processing of the amyloid-β protein precursor in mitochondria-associated membranes. *Journal of Alzheimer's Disease*. 2017;55(4):1549-1570. PMID 27911326.

Im E, Jiang Y, Stavrides PH, Darji S, Erdjument-Bromage H, Neubert TA, Choi JY, Wegiel J, Lee JH, Nixon RA. Lysosomal dysfunction in Down syndrome and Alzheimer mouse models is caused by v-ATPase inhibition by Tyr-phosphorylated APP βCTF. *Science*

Advances. 2023;9(30):eadg1925. PMID 37494443.

Kim S, Sato Y, Mohan PS, Peterhoff C, Pensalfini A, Rigoglioso A, Jiang Y, Nixon RA. Evidence that the rab5 effector APPL1 mediates APP-βCTF-induced dysfunction of endosomes in Down syndrome and Alzheimer's disease. *Molecular Psychiatry*. 2016;21(5):707-716. PMID 26194181.

Kwart D, Gregg A, Scheckel C, Murphy EA, Paquet D, Duffield M, Fak J, Olsen O, Darnell RB, Tessier-Lavigne M. A large panel of isogenic APP and PSEN1 mutant human iPSC neurons reveals shared endosomal abnormalities mediated by APP β-CTFs, not Aβ. *Neuron*. 2019;104(2):256-270.e5. PMID 31416668.

Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R. APP processing and synaptic function. *Neuron*. 2003;37(6):925-937. PMID 12670422.

Lauritzen I, Pardossi-Piquard R, Bauer C, Brigham E, Abraham JD, Ranaldi S, Fraser P, St-George-Hyslop P, Le Thuc O, Espin V, Chami L, Dunys J, Checler F. The β-secretase-derived C-terminal fragment of βAPP, C99, but not Aβ, is a key contributor to early intraneuronal lesions in triple-transgenic mouse hippocampus. *Journal of Neuroscience*. 2012;32(46):16243-16255. PMID 23152608.

Lauritzen I, Pardossi-Piquard R, Bourgeois A, Pagnotta S, Biferi MG, Barkats M, Lacor P, Klein W, Bauer C, Checler F. Intraneuronal aggregation of the β-CTF fragment of APP (C99) induces Aβ-independent lysosomal-autophagic pathology. *Acta Neuropathologica*. 2016;132(2):257-276. PMID 27138984.

Lee JH, Yang DS, Goulbourne CN, Im E, Stavrides P, Pensalfini A, Chan H, Bouchet-Marquis C, Bleiwas C, Berg MJ, Huo C, Peddy J, Pawlik M, Levy E, Rao M, Staufenbiel M, Nixon RA. Faulty autolysosome acidification in Alzheimer's disease mouse models induces autophagic build-up of Aβ in neurons, yielding senile plaques. *Nature Neuroscience*. 2022;25(6):688-701. PMID 35654956.

Montesinos J, Pera M, Larrea D, Guardia-Laguarta C, Agrawal RR, Velasco KR, Yun TD, Stavrovskaya IG, Xu Y, Koo SY, Snead AM, Sproul AA, Area-Gómez E. The Alzheimer's disease-associated C99 fragment of APP regulates cellular cholesterol trafficking. *EMBO Journal*. 2020;39(20):e103791. PMID 32865299.

Pensalfini A, Kim S, Subbanna S, Bleiwas C, Goulbourne CN, Stavrides PH, Jiang Y, Lee JH, Darji S, Pawlik M, Huo C, Peddy J, Berg MJ, Smiley JF, Basavarajappa BS, Nixon RA. Endosomal dysfunction induced by directly overactivating Rab5 recapitulates prodromal and neurodegenerative features of Alzheimer's disease. *Cell Reports*. 2020;33(8):108420. PMID 33238112.

Pera M, Larrea D, Guardia-Laguarta C, Montesinos J, Velasco KR, Agrawal RR, Xu Y, Chan RB, Di Paolo G, Mehler MF, Perumal GS, Macaluso FP, Freyberg ZZ, Acin-Perez R, Enriquez JA, Schon EA, Area-Gómez E. Increased localization of APP-C99 in mitochondria-associated ER membranes causes mitochondrial dysfunction in Alzheimer disease. *EMBO Journal*. 2017;36(22):3356-3371. PMID 29018038.

Puzzo D, Privitera L, Leznik E, Fà M, Staniszewski A, Palmeri A, Arancio O. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *Journal of Neuroscience*. 2008;28(53):14537-14545. PMID 19118188.

Song Y, Hustedt EJ, Brandon S, Sanders CR. Competition between homodimerization and cholesterol binding to the C99 domain of the amyloid precursor protein. *Biochemistry*. 2013;52(30):5051-5064. PMID 23865807.

Szaruga M, Munteanu B, Lismont S, Veugelen S, Horré K, Mercken M, Saido TC, Ryan NS, De Vos T, Savvides SN, Gallardo R, Schymkowitz J, Rousseau F, Fox NC, Hopf C, Chávez-Gutiérrez L, De Strooper B. Alzheimer's-causing mutations shift Aβ length by destabilizing γ-secretase-Aβn interactions. *Cell*. 2017;170(3):443-456.e14. PMID 28753424.

Tambini MD, Pera M, Kanter E, Yang H, Guardia-Laguarta C, Holtzman D, Sulzer D, Area-Gómez E, Schon EA. ApoE4 upregulates the activity of mitochondria-associated ER membranes. *EMBO Reports*. 2016;17(1):27-36. PMID 26564908.