THE MITOCHONDRION AS NEXUS

Function, Dysfunction, and the Bioenergetic Architecture of Neurodegeneration

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Expanded Edition

Third thesis in the Collapse trilogy, companion to Convergent Synaptic Collapse and Homeostatic Microglial Collapse. Integrating twelve research programs across mitochondrial biology, autophagy, ferroptosis, immunometabolism, and neurodegeneration.

"The mitochondrion is not merely an organelle; it is the arbiter of cellular fate, the integrator of metabolic state, and the sentinel whose failure permits every downstream catastrophe."

— Adapted from Picard & McEwen, 2018

For those whose mitochondria have already begun to fail, and for the scientists who are racing to understand why.

THE COLLAPSE TRILOGY

I. Convergent Synaptic Collapse

II. Homeostatic Microglial Collapse

III. Bioenergetic Collapse ☐ this volume

Each thesis in the trilogy addresses a different level of the same integrated model: circuit (perineuronal nets and PV^+ interneurons), cellular (microglial homeostatic identity), and substrate (mitochondrial and autophagy-lysosomal quality control). The ferroptosis-oxytosis axis, introduced in this volume, provides the lipid-peroxidation arm that connects all three levels through the v-ATPase bottleneck.

Abstract

Mitochondria occupy a singular position in neurodegenerative disease: they supply the ATP on which every synaptic event, ion gradient, protein-folding chaperone, and degradative organelle depends, and their failure generates the reactive oxygen species, damage-associated molecular patterns, and calcium dysregulation that drive each of the canonical proteinopathies. This dissertation integrates evidence across Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, and frontotemporal dementia to argue that mitochondrial quality-control failure—encompassing oxidative phosphorylation decline, mitophagy impairment, fission/fusion dysregulation, mitochondria-associated membrane disruption, NAD^+ depletion, and ferroptotic lipid peroxidation—constitutes a shared upstream substrate whose cell-type-specific manifestations determine the clinical phenotype of each disorder.

The thesis is organized in six analytical chapters. Chapter I establishes the bioenergetic architecture of the neuron, tracing the electron transport chain (Complexes I–V), the NAD^+/NADH redox couple, the TOM40 import machinery, and the mitochondria-associated membrane as an integrated functional unit. Chapter II examines how five aggregation-prone proteins each independently target mitochondrial function. Chapter III—the most substantial new contribution—presents the ferroptosis-oxytosis axis as a parallel cell-death pathway inextricable from mitochondrial dysfunction, integrating Ashley Bush's iron-dependent ferroptosis framework with Pamela Maher's oxytosis/4-HNE–v-ATPase poisoning cascade and the GPX4-selenium signaling axis. Chapter IV analyzes the immunometabolic dimension, with deep treatment of CD38-mediated NAD^+ depletion and NF-κB–APP feed-forward amplification. Chapter V examines the autophagy-lysosomal terminal pathway through Nixon's PANTHOS mechanism, v-ATPase as the ATP-lysosome coupling point, the endosomal nexus, and—in expanded treatment—Estela Area-Gomez's MAM hypothesis, which reframes AD as a γ-secretase loss-of-function disorder driven by C99 accumulation and a self-perpetuating cholesterol-C99 futile cycle at mitochondria-associated membranes. Chapter VI traces circuit-level consequences: PV^+ interneuron vulnerability, perineuronal net degradation, gamma oscillation collapse, and Li-Huei Tsai's GENUS rescue paradigm.

The dissertation concludes that mitochondrial dysfunction is not a downstream consequence of proteinopathy but a rate-limiting variable whose decline sets the threshold for disease initiation. Therapeutic implications—including mitophagy inducers, NAD^+ precursors, iron chelation, GPX4 stabilization, electron carrier bypasses, and systemic metabolic interventions—are evaluated against the

mechanistic framework. Seven falsifiable experimental predictions are generated.

Keywords: mitochondria, neurodegeneration, ferroptosis, oxidative phosphorylation, mitophagy, NAD^+, NLRP3, GPX4, 4-HNE, iron, mitochondria-associated membrane, perineuronal nets, Alzheimer's disease, Parkinson's disease

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Introduction

1.1 The Research Problem

The neurodegenerative diseases—Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and frontotemporal dementia (FTD)—are conventionally classified by their signature aggregation-prone protein: amyloid-beta and tau in AD, alpha-synuclein in PD, huntingtin in HD, and TDP-43 in ALS/FTD. This nosological framework has organized two decades of therapeutic development around aggregate clearance, yielding anti-amyloid monoclonal antibodies (lecanemab, donanemab) with statistically significant but clinically modest effects and no disease-modifying therapies for PD, ALS, HD, or FTD.

A persistent anomaly cuts across the proteinopathy framework: every one of these diseases features early, pronounced, and progressive mitochondrial dysfunction. FDG-PET hypometabolism precedes clinical AD symptoms by decades (Reiman et al., 1996; Mosconi et al., 2008). Complex I deficiency is the biochemical signature of PD substantia nigra (Schapira et al., 1989). Mutant huntingtin causes Complex II/III deficiency years before chorea onset (Gu et al., 1996). TDP-43 accumulates inside mitochondria and inhibits Complex I in ALS motor neurons (Wang et al., 2016).

This dissertation addresses the question: **What role does mitochondrial function and dysfunction play in neurodegeneration?** The thesis advanced here is that mitochondrial quality-control failure constitutes a shared upstream substrate—a bioenergetic bottleneck—whose decline sets the threshold for disease initiation and whose cell-type-specific manifestations determine which proteinopathy phenotype emerges.

A second, equally important strand of the argument concerns the ferroptosis-oxytosis axis. Ashley Bush and colleagues have argued that AD is

fundamentally a disease of iron-dependent, non-apoptotic cell death (ferroptosis) driven by progressive brain iron accumulation, where amyloid plaques and tau tangles function as compensatory sinks for toxic lipid aldehydes and redox-active iron. Pamela Maher and colleagues have mapped the biochemical cascade from glutathione depletion through 12/15-lipoxygenase activation to 4-HNE generation, demonstrating that this toxic lipid aldehyde directly poisons the lysosomal v-ATPase—providing the molecular bridge between oxidative stress and the autophagic collapse described by Nixon. This dissertation integrates the ferroptosis-oxytosis axis as a parallel, interacting cell-death pathway inextricable from mitochondrial dysfunction.

1.2 Significance

The significance of this reframing is fourfold. First, it explains the age dependence of sporadic neurodegeneration: mitochondrial function declines with age in all individuals, and inherited mitochondrial capacity determines when the decline crosses a pathogenic threshold (Swerdlow & Khan, 2004). Second, it accounts for the transdiagnostic overlap among neurodegenerative diseases as consequences of a common bioenergetic substrate whose failure permits multiple downstream cascades. Third, it identifies a therapeutic target space—mitophagy, NAD^+ metabolism, iron chelation, GPX4 stabilization, electron transport chain bypass, and systemic metabolic intervention—orthogonal to aggregate clearance and potentially complementary to it. Fourth, it provides a mechanistic explanation for the newly recognized phenomenon of cognitive resilience: individuals who maintain mitochondrial and microglial homeostasis preserve perineuronal net integrity and resist cognitive decline despite high pathological burden (de Vries et al., 2024).

1.3 Scope and Limitations

This dissertation is a synthetic review integrating twelve major research programs into a unified analytical framework. The work is biased toward AD because the mitochondrial literature is deepest in that disease, but each chapter systematically addresses PD, ALS, HD, and FTD where evidence permits. The thesis cannot resolve whether mitochondrial failure is genuinely upstream of proteinopathy or bidirectionally coupled with it; this is flagged as the central open question throughout.

2

Literature Review

2.1 Historical Foundations: Mitochondria and the Brain

The relationship between brain metabolism and cognitive function was established long before mitochondria were recognized as the site of oxidative phosphorylation. Alois Alzheimer's 1907 case report described not only neurofibrillary tangles and plaques but also "adipose inclusions" in neurons—now interpretable as lipid-laden autophagic vacuoles reflecting degradative failure (Alzheimer, 1907). Oskar Fischer, publishing contemporaneously, documented similar pathology with emphasis on the reactive glial component (Fischer, 1907, 1910). The metabolic dimension remained implicit until Warburg's characterization of aerobic glycolysis (1956) and Kennedy and Lehninger's identification of mitochondria as sites of oxidative phosphorylation (1949).

The modern study of mitochondrial dysfunction in neurodegeneration began with three parallel discoveries. Schapira et al. (1989) reported a selective 35% reduction in Complex I activity in PD substantia nigra. Langston et al.'s characterization of MPTP-induced parkinsonism (1983) demonstrated that environmental Complex I inhibition could recapitulate PD. And Parker, Filley, and Parks (1990) reported reduced cytochrome oxidase (Complex IV) activity in AD brain tissue.

The role of iron in neurodegeneration has a parallel but largely separate history. Hallervorden and Spatz described iron accumulation in basal ganglia degeneration in 1922. Goodman (1953) documented elevated iron in AD brain. Connor et al. (1992) provided systematic quantification of iron accumulation in specific AD-vulnerable regions. But it was not until Stockwell's formal definition of ferroptosis as a distinct cell-death modality in 2012 that the iron-oxidative stress literature acquired a unifying mechanistic framework.

2.2 The Mitochondrial Cascade Hypothesis

Swerdlow and Khan (2004) formalized observations of mitochondrial dysfunction in AD into the Mitochondrial Cascade Hypothesis (MCH). The MCH proposes that inherited mitochondrial function, determined primarily by maternally transmitted mtDNA, varies across the population; that age-related accumulation of somatic mtDNA mutations erodes mitochondrial function from this baseline; that when bioenergetic capacity crosses a cell-type-specific threshold, disease-associated pathologies emerge as downstream consequences; and that onset age reflects the inherited starting point rather than an exogenous trigger.

The MCH was updated in 2018 to engage with the cybrid model system, in which mitochondria from AD patients are transferred into rho-zero recipient cells, demonstrating that the bioenergetic defect is transmissible via mtDNA and produces AD-like phenotypes including increased amyloid-beta production, oxidative stress, and apoptotic vulnerability (Swerdlow, 2018). The MCH inverts the dominant causal arrow: where the amyloid cascade places A-beta as initiating event, the MCH places mitochondrial decline upstream.

2.3 The Autophagy-Lysosomal Axis

Nixon and colleagues documented massive accumulation of autophagic vacuoles in AD neurons (Nixon et al., 2005; Nixon, 2013). Lee, Yang, Nixon et al. (2022) demonstrated that autolysosomes fail to acidify, autophagic cargo accumulates, and affected neurons exhibit PANTHOS (“Poisonous Flower”)—rosette-like autolysosome expansions whose death generates plaque-like structures from the inside out. The critical coupling point is the v-ATPase proton pump: lysosomal acidification requires continuous ATP hydrolysis (Mindell, 2012). When mitochondrial ATP declines, v-ATPase fails, lysosomes de-acidify, and the entire degradative system backs up.

Nixon’s Gold Prize work established that APP-betaCTF (C99) directly inhibits v-ATPase, that Rab5 hyperactivation drives endosomal enlargement, and that PANTHOS represents a previously unrecognized mechanism of plaque formation distinct from extracellular seeding. The endosomal nexus—where multiple genetic risk factors (BIN1, PICALM, SORL1, CD2AP) converge—represents the most gene-dense convergence node in AD genetics.

2.4 Canonical Mitophagy

Narendra, Tanaka, Suen, and Youle (2008) demonstrated that Parkin is recruited to depolarized mitochondria and initiates outer-membrane ubiquitination. PINK1 was established as the upstream sensor. Loss-of-function mutations in *PINK1* or *PRKN* cause autosomal-recessive juvenile parkinsonism, establishing that mitophagy failure is sufficient for neurodegeneration (Kitada et al., 1998; Valente et al., 2004).

2.5 Mitophagy Failure in Alzheimer's Disease

Fang et al. (2019) demonstrated reduced basal mitophagy in postmortem AD hippocampus, APP/PS1 mice, and iPSC-derived AD neurons. Small-molecule mitophagy inducers (urolithin A, NMN) reduced A-beta, tau phosphorylation, and cognitive deficits. Lautrup et al. (2019) argued that age-related NAD^+ depletion is a central driver of bioenergetic failure engaging the sirtuin-PGC-1α biogenesis program.

2.6 Microglial Metabolic Reprogramming

Ulland et al. (2017) showed TREM2-DAP12-SYK signaling through PI3K-AKT-mTOR supports microglial OXPHOS. Baik et al. (2019) exposed microglia to A-beta and reported an initial glycolytic shift followed by metabolic collapse—not merely a switch but a bioenergetically unsustainable state. The Homeostatic Microglial Collapse thesis (Gustafsson, 2026) argues that loss of the TGF-β/SMAD-maintained homeostatic signature is the single upstream event from which all post-homeostatic phenotypes (DAM, LDAM, dystrophic) emerge as context-dependent collapse trajectories.

2.7 NLRP3 Inflammasome

Heneka et al. (2013) demonstrated that *Nlrp3* deletion reduces AD pathology in APP/PS1 mice. Venegas et al. (2017) showed ASC specks cross-seed A-beta aggregation. Ising et al. (2019) extended this to tau. Mitochondrial DAMPs (mtROS, oxidized mtDNA, cardiolipin) are among the best-characterized NLRP3 activators.

2.8 Ferroptosis and the Oxytosis Pathway

Dixon et al. (2012) formally defined ferroptosis as a regulated, non-apoptotic cell-death modality driven by iron-dependent accumulation of lipid peroxides. The pathway is initiated when GPX4 (glutathione peroxidase 4) fails to neutralize lipid hydroperoxides, allowing unchecked oxidation of polyunsaturated fatty acids (PUFAs) in neuronal membranes. Stockwell and colleagues systematically characterized the molecular machinery: erastin inhibits System X_c^- (cystine/glutamate antiporter), depleting intracellular cysteine and glutathione, leading to GPX4 inactivation and iron-catalyzed lipid peroxidation (Yang et al., 2014).

Ashley Bush and colleagues reframed AD through the ferroptosis lens: age-dependent brain iron accumulation fuels Fenton chemistry; APP stabilizes ferroportin for iron efflux, and familial AD mutations impair this function, causing iron retention; presenilin-1 mutations disrupt a Notch-LRP8-GPX4 signaling axis that sustains selenium uptake for GPX4 synthesis; and both amyloid plaques and tau tangles may function as compensatory sinks that sequester toxic lipid aldehydes and redox-active iron (Ayton et al., 2015, 2020; Kenkhuis et al., 2023). Bush's 2025 *Brain* review—"Iron on trial"—engaged critically with the failed deferiprone clinical trial and argued that iron is vital for brain health; the therapeutic target is iron dyscompartmentalization, not iron reduction.

Pamela Maher and David Schubert developed the oxytosis/ferroptosis hypothesis, mapping the pathway from System X_c^- inhibition through 12/15-lipoxygenase (12/15-LOX) activation to generation of 4-hydroxynonenal (4-HNE), a toxic lipid aldehyde that covalently modifies and inhibits the lysosomal v-ATPase proton pump (Lewerenz et al., 2018; Maher, 2020). This 4-HNE–v-ATPase poisoning provides the biochemical bridge between oxidative stress and the autophagic collapse described by Nixon. Maher's compounds J147 and CMS121 rescue mitochondrial function and prevent ferroptotic cell death in AD models through AMPK/mTOR-dependent autophagy restoration and FASN inhibition.

2.9 Mitochondrial Allostatic Load and Mitohormesis

Picard, Juster, and McEwen (2014) introduced mitochondrial allostatic load. Schulz et al. (2007) demonstrated mitohormesis: caloric restriction extends lifespan through transient ROS elevation that triggers NRF2 and AMPK-PGC-1α biogenesis. The metabolic psychiatry literature (Sethi et al., 2026) reframes systemic insulin resistance and mitochondrial dysfunction as a transdiagnostic substrate spanning schizophrenia, bipolar disorder, depression, and neurodegeneration.

2.10 Gaps in the Literature

Five significant gaps persist. First, the mitochondrial and autophagy-lysosomal literatures have developed in isolation from microglial biology. Second, the CD38-NAD^+ axis in microglial bioenergetic collapse has not been connected to neurodegeneration-specific phenotypes. Third, ferroptosis has been studied primarily as a cell-death endpoint without systematic integration into the bioenergetic collapse framework. Fourth, environmental neurotoxin literature has been treated as disease-specific. Fifth, the perineuronal net—the single most concrete readout for the entire synthesis—has not been systematically linked to upstream bioenergetic variables. This dissertation addresses all five gaps.

3

Methodology

This dissertation adopts a systems-level integrative review methodology synthesizing primary experimental literature, clinical trial data, and theoretical frameworks across neuroscience, immunology, metabolism, and pharmacology. Primary sources were selected for publication in peer-reviewed journals, adequate experimental methodology, relevance to twelve core research programs, and recency (post-2015 except foundational work).

A total of 135 Oskar Fischer Prize entrant submissions (2020) were evaluated using the Synthesis-Relevance Rescore system against a registry of 109 Tier-1 and 126 Tier-2 load-bearing mechanisms across the Collapse trilogy (Gustafsson, 2026). The analysis proceeds through four integration levels: **organelle** (mitochondrial architecture and coupling), **pathway** (ferroptosis-oxytosis as parallel cell-death modality), **cellular** (microglial homeostatic collapse, astrocytic metabolic support), and **circuit** (PV^+ interneurons, perineuronal nets, gamma oscillations). Citations follow APA 7th edition.

Chapter I

The Bioenergetic Architecture of the Neuron

4.1 The Electron Transport Chain: Complexes I–V

Complex I (NADH:ubiquinone oxidoreductase) accepts electrons from NADH and pumps four protons per NADH oxidized. It is the largest ETC complex (~45 subunits) and the primary site of electron leak generating superoxide (Brand, 2010). Complex I is the convergent target of rotenone, MPTP/MPP^+, annonacin, alpha-synuclein, and TDP-43. **Complex II (succinate dehydrogenase)**, the only entirely nuclear-encoded ETC complex, is selectively impaired in Huntington's disease (Gu et al., 1996). **Complex III (cytochrome bc_1)** is the second major superoxide source. **Cytochrome c** shuttles electrons between III and IV but, upon release to cytoplasm, triggers apoptosis through caspase-9—the molecular switch where bioenergetic failure becomes cell death (Liu et al., 1996).

Complex IV (cytochrome c oxidase) is consistently reduced 25–40% in AD brain and platelets (Parker et al., 1990; Mutisya et al., 1994). Swerdlow's cybrid studies demonstrated transmissibility via mtDNA (Swerdlow et al., 1997). **Complex V (ATP synthase)** can reverse under severe depolarization, hydrolyzing ATP—converting the generator into a consumer (Chinopoulos & Adam-Vizi, 2010). **Cytochrome P450** enzymes CYP46A1 (cholesterol 24-hydroxylase) and CYP27A1 connect mitochondrial cholesterol metabolism to neuronal lipid homeostasis and oxysterol-mediated neuroinflammation.

4.2 The Energy Currency: NAD^+/NADH and the CD38 Problem

NAD^+ is the electron carrier feeding Complex I and the essential co-substrate for sirtuins, PARPs, and CD38/CD157. NAD^+ depletion simultaneously cripples OXPHOS, mitochondrial biogenesis (sirtuin-PGC-1α failure), and DNA repair (Verdin, 2015). Camacho-Pereira et al. (2016) identified CD38 as the dominant NAD^+-consuming enzyme in aging tissue. CD38 is highly expressed on activated microglia—microglial activation upregulates the very enzyme that depletes the NAD^+ required for OXPHOS. The NAD^+/NADH ratio in stroke provides the acute-crisis parallel: ischemia causes catastrophic NAD^+ depletion through PARP1 hyperactivation.

4.3 The Gateway: TOM40 and Mitochondrial Protein Import

Over 99% of mitochondrial proteins enter through TOM40 (Wiedemann & Pfanner, 2017). The *TOMM40* gene lies in linkage disequilibrium with *APOE* on chromosome 19q13.32. Roses et al. (2010) identified a poly-T repeat polymorphism correlating with AD onset age. Amyloid-beta itself is imported through TOM40 into the matrix, where it inhibits Complex IV (Hansson Petersen et al., 2008).

4.4 The Contact Site: Mitochondria-Associated Membrane (MAM)

The MAM is a specialized ER subdomain tethered to the outer mitochondrial membrane (Csordás et al., 2018). **Calcium transfer**: IP3R–GRP75–VDAC–MCU couples ER calcium release to mitochondrial uptake, stimulating TCA cycle dehydrogenases (Rizzuto et al., 2012). **BAP31**: ER-resident chaperone whose cleavage generates pro-apoptotic p20BAP31, promoting calcium release into mitochondria. **ABAD** (HSD17B10): matrix enzyme that A-beta binds directly, distorting its NAD^+-binding site and amplifying ROS (Lustbader et al., 2004). **Lipid metabolism**: Area-Gomez et al. (2012) showed C99 accumulates at MAMs as a cholesterol sensor, driving aberrant cholesterol esterification and phospholipid synthesis—disrupting both mitochondrial membrane composition and ER lipid homeostasis.

Area-Gomez's Gold Prize work reframed AD as fundamentally a lipid disorder: C99 signals false cholesterol deficiency at MAMs, driving ACAT1-mediated cholesterol esterification that creates hydrophobic mismatch favoring A-beta42 production. The dual C99 toxicity—through both lipid disruption and v-ATPase inhibition—is one of the most mechanistically integrative findings in the field. Lipidomic signatures are detectable in asymptomatic PSEN1-E280A carriers as young as age 6–12, placing lipid dysfunction decades before any other measurable biomarker.

4.5 Calcium: The Universal Coupling Signal

Neurons maintain cytosolic calcium at ~100 nM against 2 mM extracellular—a 20,000-fold gradient requiring ATP-dependent pumps (SERCA, PMCA, MCU). Khachaturian's Calcium System Theory (CAST-AD) frames AD as progressive degradation of neuronal calcium control, modeled through cybernetic control theory as a phase transition from functional to degenerative attractor states (Khachaturian, 1987, 1989). Elevated cytosolic calcium activates calpain, which cleaves v-ATPase subunits—directly connecting calcium dysregulation to lysosomal acidification failure. Presenilin mutations dysregulate ER calcium stores through ryanodine and IP3 receptors (Tu et al., 2006). The terminal step in the oxytosis pathway is store-operated calcium entry (SOCE) through Orai1 channels, leading to cell lysis (Maher, 2020).

4.6 Mitochondrial Quality Control: Mitophagy and Fission/Fusion

Fusion (MFN1/2, OPA1) rescues mildly damaged mitochondria by functional complementation. **Fission** (DRP1, FIS1) segregates irreparably damaged segments for mitophagy disposal (Youle & van der Bliek, 2012). **Ubiquitin** is the molecular tag: Parkin writes polyubiquitin chains read by autophagy receptors (p62, OPTN, NDP52) and edited by deubiquitinases (USP30, USP15). The endolysosomal system is the terminal executor—autophagosomes must fuse with lysosomes for degradation, requiring v-ATPase acidification (ATP-dependent), functional cathepsins (pH-dependent), and SNARE fusion machinery. When any fails, mitophagy stalls.

4.7 Energy Production and Reactive Oxygen Species

ETC electron leak at Complex I and III generates superoxide. At low levels, ROS signal adaptively—NRF2 activation, mitohormesis (Ristow & Schmeisser, 2014). The pathological transition occurs when damage exceeds repair: oxidized mtDNA mutations impair the ETC further (the vicious cycle: Linnane et al., 1989), and released mtDNA fragments activate cGAS-STING and NLRP3. **The relationship between mitochondrial ROS and ferroptosis is direct**: mitochondrial superoxide reacts with labile iron via Fenton chemistry to generate hydroxyl radicals that attack membrane PUFAs, initiating the lipid peroxidation cascade that ferroptosis describes. Mitochondria are therefore not merely bystanders in ferroptosis—they are its primary ROS source and its primary iron reservoir.

4.8 Synapse Function and GABAergic Vulnerability

Synaptic transmission is the most energy-intensive brain process. **PV^+ fast-spiking interneurons** fire at gamma frequency (30–80 Hz) continuously, imposing the highest per-neuron ATP demand (Kann et al., 2014). They are ensheathed by perineuronal nets that stabilize synapses, buffer ions, chelate iron, and protect against oxidative stress (Cabungcal et al., 2013). PV^+ interneuron loss is an early AD feature (Verret et al., 2012). Li-Huei Tsai's GENUS paradigm demonstrates that 40 Hz sensory stimulation restores mitochondrial ATP production, microglial phagocytosis, and glymphatic clearance (Iaccarino et al., 2016).

4.9 Environmental Toxins as Natural Experiments

Annonacin (Complex I inhibitor from Annonaceae fruits) produces PSP-like tauopathy without amyloid (Champy et al., 2004; Höglinger et al., 2005). **BMAA** (cyanobacterial toxin) inhibits Complex I, generates ROS, produces TDP-43 aggregation and neurofibrillary tangles with plaques in primates (Cox et al., 2016). **Rotenone** produces parkinsonism with alpha-synuclein aggregation. These natural experiments demonstrate that mitochondrial poisoning alone is sufficient to produce the full neuropathological spectrum—the strongest evidence for the upstream interpretation.

Chapter II

Disease Proteins as Mitochondrial Toxins

5.1 Amyloid-Beta

A-beta interacts with mitochondria at multiple points: matrix import via TOM40 with Complex IV inhibition (Hansson Petersen et al., 2008); ABAD binding with ROS amplification (Lustbader et al., 2004); MAM disruption with calcium overload (Hedskog et al., 2013); mitophagy impairment through PINK1/Parkin interference (Fang et al., 2019); and NF-κB activation driving APP transcription (Grilli et al., 1995)—creating the feed-forward loop: **mitochondrial damage ☐ NF-κB ☐ APP ☐ A-beta ☐ more mitochondrial damage**.

In Bush's ferroptosis framework, A-beta has an additional role: APP stabilizes ferroportin (the sole cellular iron exporter), and A-beta itself binds iron with high affinity, potentially sequestering redox-active iron in plaques. This reframes plaques not merely as waste deposits but as iron-chelating structures that may initially serve a protective function—absorbing the ferroptotic threat. The protective reframing aligns with the observation that plaque burden correlates poorly with cognitive decline; what matters is the ratio of sequestered to unsequestered iron.

5.2 Tau

Hyperphosphorylated tau disrupts axonal mitochondrial transport (Dixit et al., 2008; Kopeikina et al., 2011). Truncated tau directly inhibits Complex I (Quintanilla et al., 2009). The relationship is bidirectional: Complex I inhibition by annonacin causes tau hyperphosphorylation (Höglinger et al., 2005). In the ferroptosis framework, neurofibrillary tangles may function as 4-HNE sinks—tau's abundant lysine residues are targets for 4-HNE Michael addition, and sequestering this toxic aldehyde may be an early compensatory response that becomes pathological only when overwhelmed.

5.3 Alpha-Synuclein

Alpha-synuclein oligomers inhibit Complex I (Devi et al., 2008), localize to MAMs disrupting calcium signaling (Guardia-Laguarta et al., 2014), impair mitophagy through cardiolipin sequestration (Shaltouki et al., 2018), and promote DRP1-mediated fragmentation (Nakamura et al., 2011). Alpha-synuclein also binds iron, and iron-loaded alpha-synuclein is more prone to aggregation (Binolfi et al., 2006)—connecting PD pathology to the ferroptosis axis.

5.4 Mutant Huntingtin

Mutant huntingtin selectively impairs Complex II (Benchoua et al., 2006), represses PGC-1α transcription (Cui et al., 2006), promotes DRP1-mediated fission (Song et al., 2011), and sensitizes IP3 receptors at the ER (Tang et al., 2003). The Complex II selectivity may explain HD's striatal targeting.

5.5 TDP-43

TDP-43 is imported into the inner mitochondrial membrane and directly inhibits Complex I by binding ND3 and ND6 subunits (Wang et al., 2016). Blocking mitochondrial import prevents neurodegeneration in animal models—demonstrating that a nuclear RNA-binding protein has a direct, independent mitochondrial toxicity mechanism.

5.6 Synthesis: The Protein-Convergent Mitochondrial Hypothesis

Every disease protein independently poisons the ETC with partial complex selectivity—Complex I (A-beta, alpha-synuclein, TDP-43), Complex II (huntingtin), Complex IV (A-beta)—and all impair quality control. Environmental toxins demonstrate that mitochondrial poisoning alone suffices to produce neurodegeneration with proteinopathy. The strong interpretation: age-related mitochondrial decline creates the vulnerable substrate; disease proteins are additional stressors catastrophic only because they impinge on an already-compromised system.

Chapter III

Ferroptosis and the Oxidative-Lipid Axis

Iron, GPX4, 4-HNE, and the Biochemical Bridge to Autophagic Collapse

6.1 Ferroptosis: Definition and Molecular Machinery

Ferroptosis was formally defined by Dixon et al. (2012) as a regulated form of non-apoptotic cell death driven by iron-dependent accumulation of lipid peroxides in cellular membranes. The pathway is morphologically, biochemically, and genetically distinct from apoptosis, necrosis, and autophagy. Ferroptotic cells display shrunken mitochondria with increased membrane density, reduced cristae, and ruptured outer membrane—but without the chromatin condensation of apoptosis or the organelle swelling of necrosis (Xie et al., 2016).

The core molecular machinery operates through four interacting systems:

- **The glutathione-GPX4 axis**: System X_c^- (SLC7A11/SLC3A2) imports extracellular cystine in exchange for intracellular glutamate. Cystine is reduced to cysteine, the rate-limiting substrate for glutathione (GSH) synthesis. GPX4 uses GSH to reduce phospholipid hydroperoxides (PL-OOH) to their corresponding alcohols (PL-OH), neutralizing the lipid peroxidation chain reaction. GPX4 is the only enzyme capable of reducing membrane-integrated lipid peroxides (Ursini et al., 1982; Yang et al., 2014).
- **Iron-catalyzed Fenton chemistry**: Labile ferrous iron (Fe^{2+}) reacts with hydrogen peroxide to generate hydroxyl radicals (•OH) that attack polyunsaturated fatty acids in membranes. The brain is iron-rich (50–60 mg total in adult brain), and iron accumulates further with aging, particularly in hippocampus, substantia nigra, and basal ganglia (Zecca et al., 2004).
- **Lipoxygenase-mediated peroxidation**: 12/15-lipoxygenase (ALOX15) enzymatically oxidizes arachidonic acid-containing phosphatidylethanolamine

(AA-PE) in membranes. This enzymatic pathway is faster and more spatially targeted than Fenton chemistry (Kagan et al., 2017).

• **The FSP1-CoQ$_{10}$ parallel pathway**: Ferroptosis suppressor protein 1 (FSP1, also AIFM2) reduces coenzyme Q$_{10}$ to ubiquinol, which acts as a radical-trapping antioxidant in membranes independently of GPX4 (Doll et al., 2019; Bersuker et al., 2019). This represents a GPX4-independent anti-ferroptotic defense.

The discovery that the brain's primary antioxidant defense (GPX4) depends on selenium uptake, that selenium uptake depends on LRP8 receptor expression, and that LRP8 expression is regulated by Presenilin-1/Notch signaling creates a direct genetic link from familial AD mutations to ferroptotic vulnerability—a link discovered by Bush and colleagues and described below.

6.2 Bush: The Ferroptosis Theory of Alzheimer's Disease

Ashley Bush and colleagues have developed the most comprehensive ferroptosis-centered framework for AD. The theory rests on several interlocking claims, each supported by specific evidence:

6.2.1 Age-Dependent Brain Iron Accumulation

Brain iron increases with age in regions selectively vulnerable to AD—hippocampus, temporal cortex, and basal forebrain (Bartzokis et al., 2007; Raven et al., 2013). MRI-based iron quantification (QSM, R2*) shows that higher hippocampal iron predicts faster cognitive decline in preclinical AD (Ayton et al., 2020). Postmortem studies confirm that AD brains have elevated ferritin-bound and labile iron in hippocampal neurons and plaques (Goodman, 1953; Connor et al., 1992; Lovell et al., 1998).

The source of iron accumulation is debated. One hypothesis is that blood-brain barrier breakdown permits peripheral iron entry. A second—favored by Bush—is that APP-mediated iron efflux fails: APP stabilizes the iron exporter ferroportin on the cell surface, promoting iron export. Familial AD mutations in APP and PSEN1 reduce surface APP and therefore reduce ferroportin-mediated iron efflux, causing intracellular iron retention (Duce et al., 2010; Wong et al., 2014). A-beta peptides themselves bind iron with high affinity; amyloid plaques contain substantial iron

deposits.

6.2.2 The Presenilin-Notch-LRP8-GPX4 Signaling Axis

Bush and colleagues identified a signaling pathway linking presenilin-1 to GPX4 expression: PSEN1 cleaves Notch-1 to release the Notch intracellular domain (NICD), which translocates to the nucleus and activates transcription of LRP8 (also called ApoER2). LRP8 is the receptor for Selenoprotein P, the primary selenium delivery protein to the brain. Selenium is required for selenocysteine incorporation into GPX4's active site—without selenium, GPX4 is catalytically dead.

Familial AD mutations in PSEN1 impair Notch cleavage, reducing NICD, reducing LRP8 expression, reducing selenium uptake, and therefore reducing functional GPX4—leaving neurons defenseless against lipid peroxidation. This signaling chain (PSEN1 ☐ Notch ☐ NICD ☐ LRP8 ☐ Selenoprotein P uptake ☐ selenium ☐ GPX4) provides the first direct genetic mechanism linking familial AD mutations to ferroptotic vulnerability. It reframes PSEN1 mutations as loss-of-function events that impair anti-ferroptotic defenses, rather than gain-of-function events that increase toxic A-beta42 production.

6.2.3 Plaques and Tangles as Ferroptotic Sinks

The most provocative element of Bush's framework is the reinterpretation of amyloid plaques and neurofibrillary tangles as compensatory structures. Plaques sequester redox-active iron through A-beta's iron-binding capacity, reducing labile iron available for Fenton chemistry. Tangles sequester 4-HNE through Michael addition to tau's abundant lysine residues, reducing the 4-HNE available to poison v-ATPase and other protein targets. In this framing, plaques and tangles are initially protective—they become pathological only when the ferroptotic burden overwhelms their sequestration capacity, or when the aggregation itself produces secondary toxicity through space-occupying and inflammatory effects.

This interpretation is consistent with several otherwise puzzling observations: (a) many cognitively normal elderly individuals have substantial plaque and tangle burden; (b) plaque burden correlates poorly with cognitive decline; (c) anti-amyloid therapies reduce plaques but produce only modest cognitive benefit; (d) iron-rich plaques may be the most pathologically significant subpopulation. The framework predicts that iron status, not aggregate quantity, should be the stronger predictor of

clinical progression.

6.3 Maher: Oxytosis/Ferroptosis and the 4-HNE–v-ATPase Bridge

Pamela Maher and David Schubert developed the oxytosis/ferroptosis hypothesis, which maps the complete biochemical cascade from initial insult to terminal cell death (Tan et al., 2001; Lewerenz et al., 2018; Maher, 2020):

6.3.1 The Oxytosis/Ferroptosis Cascade

Step 1: Glutathione depletion. Extracellular glutamate (elevated in AD from synaptic spillover, reduced reuptake, and microglial release) inhibits System X_c^-, blocking cystine import and depleting intracellular cysteine and GSH. Aging itself reduces System X_c^- expression.

Step 2: GPX4 inactivation. Without GSH substrate, GPX4 cannot reduce lipid peroxides. GPX4 protein is depleted in AD brain (Yoo et al., 2010). 12/15-lipoxygenase (12/15-LOX), the enzymatic lipid peroxidase, is upregulated in AD and its inhibition is neuroprotective.

Step 3: Lipid peroxidation and 4-HNE generation. Unchecked oxidation of arachidonic acid-containing membrane phospholipids generates 4-hydroxynonenal (4-HNE), a highly reactive α,β-unsaturated aldehyde. 4-HNE is elevated in AD brain, CSF, and plasma (Markesbery & Lovell, 1998; Reed et al., 2008).

Step 4: 4-HNE poisons v-ATPase. This is Maher's most consequential finding for the broader synthesis. 4-HNE covalently modifies cysteine residues on v-ATPase subunits through Michael addition, inhibiting the proton pump and de-acidifying lysosomes. This directly connects ferroptotic lipid peroxidation to the autophagic collapse described by Nixon: 4-HNE ☐ v-ATPase inhibition ☐ lysosomal de-acidification ☐ autophagic backlog ☐ PANTHOS. The biochemical bridge is 4-HNE.

Step 5: Mitochondrial dysfunction amplification. 4-HNE also damages mitochondrial membrane lipids and proteins, inhibiting ETC complexes and generating more ROS—a feed-forward amplification loop. Failed mitophagy (because

lysosomes cannot degrade mitophagosomes) produces accumulation of damaged mitochondria generating more ROS and more 4-HNE.

Step 6: Terminal calcium entry and lysis. The cascade terminates in store-operated calcium entry (SOCE) through Orai1 channels at the plasma membrane, causing catastrophic calcium influx and cell lysis (oxytosis endpoint) or iron-dependent membrane rupture (ferroptosis endpoint). Maher has argued that oxytosis and ferroptosis represent overlapping descriptions of the same terminal pathway, distinguished primarily by the historical context in which they were characterized.

6.3.2 Therapeutic Compounds: J147, CMS121, and Fisetin

Maher's laboratory has developed several compounds that intervene at different points in the cascade. **J147** is an orally active, blood-brain-barrier-penetrant compound that enhances mitochondrial function through AMPK activation and ATP synthase modulation; it rescues cognitive deficits in aged AD mice and is in clinical development (Chen et al., 2011; Goldberg et al., 2018). **CMS121** inhibits fatty acid synthase (FASN), reducing the substrate pool for lipid peroxidation, and protects against ferroptotic cell death in AD models (Ates et al., 2020). **Fisetin** is a naturally occurring flavonoid with anti-ferroptotic, anti-inflammatory, and senolytic properties that reduces AD pathology and cognitive deficits in multiple mouse models (Currais et al., 2018). All three compounds engage the mitochondrial-ferroptotic axis rather than targeting amyloid directly.

6.4 The GPX4-Selenium Signaling Axis

GPX4 is a selenoprotein: its active site contains selenocysteine (Sec), the 21st amino acid, which is 1000-fold more catalytically efficient than cysteine at reducing lipid peroxides. Selenium bioavailability is therefore rate-limiting for GPX4 function. Brain selenium declines with age (Cardoso et al., 2010), and epidemiological studies associate low selenium status with cognitive decline and AD risk (Varikasuvu et al., 2019).

The delivery pathway is specific: Selenoprotein P (SELENOP) is the primary selenium carrier in blood and CSF. It binds to LRP8/ApoER2 on neuronal surfaces for receptor-mediated endocytosis. Once internalized, selenium is liberated and

incorporated into GPX4 through the specialized selenocysteine insertion machinery (SECIS element in GPX4 mRNA, SBP2, EFSec). Any disruption along this chain—reduced SELENOP production (liver disease, aging), reduced LRP8 expression (PSEN1 mutations, as in Bush's framework), or impaired selenocysteine insertion—produces functional GPX4 deficiency and ferroptotic vulnerability.

6.5 Iron Dyshomeostasis and the Brain Iron Paradox

Iron is essential for mitochondrial function: it is a cofactor in all four ETC complexes, in aconitase (TCA cycle), in ribonucleotide reductase (DNA synthesis), and in cytochrome P450 enzymes. The brain cannot function without iron. Yet excess labile iron catalyzes the very Fenton chemistry that destroys mitochondrial membranes and initiates ferroptosis. This is the **brain iron paradox**: the organ most dependent on iron is also most vulnerable to iron toxicity.

Iron homeostasis operates through a carefully regulated system: transferrin-bound iron enters cells via transferrin receptor 1 (TfR1); intracellular iron is stored in ferritin; iron is exported by ferroportin (the sole cellular iron exporter), whose surface expression is regulated by hepcidin. In AD, this system fails at multiple points: TfR1 is upregulated (increased import), ferritin is dysregulated (reduced safe storage), ferroportin is destabilized (reduced export through APP loss-of-function), and ferritinophagy (autophagic degradation of ferritin) releases stored iron into the labile pool precisely when the cell's antioxidant capacity is diminished.

Bush's 2025 *Brain* review—"Iron on trial"—engaged critically with the failed deferiprone clinical trial (FAIRPARK-II in PD showed worsening, not improvement). Bush argued that systemic iron chelation is counterproductive because neurons need iron; the therapeutic target is not iron reduction but iron recompartmentalization—moving labile iron back into ferritin and out of the mitochondrial matrix. This distinction has profound implications for therapeutic strategy.

6.6 APOE4 and Lipid Peroxidation Vulnerability

APOE4, the strongest genetic risk factor for sporadic AD, amplifies ferroptotic vulnerability through a specific structural mechanism. APOE2 and APOE3 contain a cysteine residue at position 112 that can form a disulfide bridge with the PUFA cargo they transport, protecting those PUFAs from oxidation. APOE4 has an arginine at position 112, eliminating the disulfide bridge and leaving its PUFA cargo unprotected against peroxidation (Huebbe & Rimbach, 2017). This structural difference means APOE4 carriers transport oxidation-vulnerable lipid cargo throughout the brain, seeding ferroptotic susceptibility at every lipid delivery site. Additionally, APOE4 causes "hypolipidation"—structural inability to accept lipids from ABCA1—starving endolysosomal membranes and destabilizing v-ATPase assembly.

6.7 Amyloid and Tau as Ferroptotic Sinks

The reinterpretation of AD's signature pathologies as compensatory responses to ferroptotic threat is the most intellectually challenging element of the ferroptosis framework. The evidence is circumstantial but convergent: A-beta binds iron and copper with high affinity and can reduce Cu^{2+} to Cu^{+}; plaques contain substantial iron deposits visible on MRI; tau's lysine residues are preferential targets for 4-HNE Michael addition; and both plaques and tangles correlate poorly with cognitive decline when iron status is controlled for. The framework predicts that interventions reducing plaque burden without addressing iron status may paradoxically worsen iron-mediated toxicity by liberating sequestered iron.

6.8 Convergence: Ferroptosis Meets Bioenergetic Collapse

Ferroptosis and bioenergetic collapse are not separate pathways—they are coupled through at least four mechanistic links:

- **Mitochondria as the primary ROS source for Fenton chemistry:** ETC electron leak generates the superoxide that, after conversion to H_2O_2, reacts with labile iron to initiate lipid peroxidation.
- **4-HNE as the biochemical bridge to lysosomal failure:** Ferroptotic lipid peroxidation generates 4-HNE, which poisons v-ATPase, which requires ATP from mitochondria—a convergent assault on the same target from two

directions.

• **Iron in the mitochondrial matrix:** Mitochondria are the cell's largest iron consumers (for Fe-S cluster and heme synthesis) and therefore its largest labile iron repository. Mitochondrial damage releases this iron directly into a ROS-rich environment.

• **GSH depletion cripples both defenses simultaneously:** GSH is required for both GPX4-mediated lipid peroxide neutralization and for glutathione peroxidase-mediated H_2O_2 detoxification in the mitochondrial matrix.

The integrated model is therefore: **mitochondrial decline ☐ increased ROS + labile iron release ☐ Fenton chemistry ☐ lipid peroxidation ☐ 4-HNE ☐ v-ATPase poisoning ☐ lysosomal failure ☐ mitophagy stalling ☐ more damaged mitochondria ☐ more ROS and iron**. Ferroptosis is not an alternative to bioenergetic collapse; it is the lipid-peroxidation arm of the same catastrophic cycle.

Chapter IV

The Immunometabolic Dimension

7.1 Mitochondrial DAMPs and Innate Immunity

Mitochondria retain bacterial signatures that the innate immune system recognizes as danger signals: mtDNA (TLR9, cGAS), cardiolipin (NLRP3), mtROS (NLRP3 via TXNIP), N-formyl peptides (formyl peptide receptors), and extracellular ATP (P2X7). Mitochondrial damage, regardless of cause, generates a stereotyped innate immune response (Zhang et al., 2010; West et al., 2015).

7.2 The NLRP3 Inflammasome as Mitochondrial-ROS-Gated Amplifier

Mitochondrial dysfunction provides both signals for NLRP3 activation: ROS-mediated NF-κB priming and mtDAMP-mediated activation. Heneka et al. (2013) showed *Nlrp3* deletion rescues AD mice. Venegas et al. (2017) demonstrated ASC speck cross-seeding of A-beta—feed-forward amplification. Ising et al. (2019) extended this to tau through GSK-3β activation. The prediction: upstream mitophagy restoration should reduce NLRP3 by preventing DAMP accumulation.

7.3 NF-κB: Feed-Forward Amplification with APP

NF-κB is activated by mtROS (IKK oxidation), mtDNA (cGAS-STING cross-activation), and IL-1β (paracrine amplification). The human APP promoter contains NF-κB binding sites (Grilli et al., 1995)—mitochondrial dysfunction drives APP transcription and A-beta production. The loop: **mitochondrial damage ☐ ROS ☐ NF-κB ☐ APP ☐ A-beta ☐ more mitochondrial damage**. This converts gradual bioenergetic decline into an accelerating cascade.

7.4 CD38 and the NAD^+ Drain

CD38 creates a devastating metabolic trap: activation upregulates CD38 ☐ NAD^+ depletion ☐ OXPHOS failure ☐ more mtDAMPs ☐ more activation ☐ more CD38. Chini et al. (2020) showed CD38 inhibition rescues NAD^+ and mitochondrial function in aged mice. Covarrubias et al. (2020) identified the SASP–CD38 axis in tissue-resident macrophages. Eduardo Chini's work (Prize #127; Trilogy score: 13) was the most significant blind spot in the CSC framework—directly load-bearing for both Bioenergetic Collapse and Homeostatic Microglial Collapse.

7.5 Inflammatory T-Cell Infiltration and Metabolic Competition

Gate et al. (2020) demonstrated clonally expanded $CD8^+$ T cells in AD CSF. Infiltrating T cells are highly glycolytic (consuming local glucose), express CD38 (NAD^+ consumption), secrete IFN-γ (reprogramming microglial metabolism toward glycolysis), and their perforin/granzymes directly damage mitochondria. Gate's work (#139; Trilogy: 15) was underscored because correct mechanisms were buried under a viral etiology narrative.

7.6 Complement Activation and Synaptic Stripping

Damaged mitochondria expose complement-activating molecules. C1q and C3 tag bioenergetically weakened synapses for microglial phagocytic elimination (Stevens et al., 2007; Hong et al., 2016)—reactivating a developmental pruning program in the adult brain. Beth Stevens' work (#158; Trilogy: 12) represents the most important validation for the connection between microglial homeostatic failure and synaptic vulnerability.

Chapter V

The Autophagy-Lysosomal Terminal Pathway

8.1 v-ATPase: The ATP-Lysosome Coupling Point

The vacuolar ATPase is a rotary proton pump that maintains lysosomal pH ~4.5 through continuous ATP hydrolysis. It is the single most important molecular link between mitochondrial bioenergetics and degradative function. v-ATPase failure converges from three directions: (a) ATP depletion from mitochondrial decline (Swerdlow); (b) direct inhibition by APP-betaCTF/C99 (Nixon); (c) covalent modification by 4-HNE from lipid peroxidation (Maher). Each is independently documented; their convergence on the same target explains why lysosomal failure is so robust and early in AD.

A fourth route of v-ATPase impairment comes through calcium: elevated cytosolic calcium activates calpain, which cleaves v-ATPase subunits (Bhatt et al., 2014). Moosmann's Silver Prize work adds a fifth: NMDA receptor hypofunction deprives neurons of the calcium/cAMP/PKA signaling needed for v-ATPase assembly, contributing from the excitatory insufficiency direction. The convergence of five independent mechanisms on a single molecular target is among the strongest evidence that v-ATPase failure is a central, rate-limiting event in AD.

8.2 PANTHOS: Inside-Out Plaque Formation

Lee et al. (2022) demonstrated that when v-ATPase fails and lysosomes de-acidify, autophagic vacuoles balloon with undegraded cargo including A-beta, eventually forming rosette-like structures (PANTHOS) that rupture the neuron. The extruded material resembles an amyloid plaque—but it formed from the inside out, not from extracellular seeding. PANTHOS does not exclude extracellular seeding; both mechanisms likely contribute. But PANTHOS provides a direct molecular pathway from autophagic failure to plaque pathology that does not require extracellular A-beta as the initiating event.

8.3 The Endosomal Nexus

The endosomal system is the most gene-dense convergence node in AD genetics. BIN1, PICALM, SORL1, CD2AP, and RAB5 risk variants all affect endosomal trafficking, sorting, and recycling. Nixon showed that Rab5 hyperactivation drives endosomal enlargement, the earliest morphological abnormality in AD neurons. The endosomal system receives cargo from the plasma membrane (receptor-mediated endocytosis), from the Golgi (biosynthetic pathway), and from autophagosomes (degradative pathway)—making it the central traffic hub whose dysfunction cascades in all directions.

8.4 Lipid Metabolism and MAM Dysfunction

Area-Gomez's framework positions AD as fundamentally a lipid disorder. C99 at MAMs signals false cholesterol deficiency, driving ACAT1-mediated esterification that changes membrane composition. APOE4 hypolipidation starves endolysosomal membranes. Ceramide dysregulation stabilizes BACE1 and activates PP2A for tau phosphorylation via GSK-3β. Lipid-droplet-accumulating microglia (LDAM; Marschallinger et al., 2020) lose phagocytic capacity and release pro-inflammatory cytokines—connecting lipid failure in microglia to the homeostatic collapse described in the companion thesis.

8.5 Ubiquitin: The Shared Quality-Control Language

Ubiquitin tags both misfolded proteins (for proteasomal and autophagic degradation) and damaged mitochondria (for mitophagy). When proteotoxic stress from aggregation-prone proteins overwhelms the UPS, excess cargo routes to autophagy—already overloaded with mitophagic cargo. The result: competition for limited degradative capacity (Rubinsztein, 2006; Dikic, 2017). David Rubinsztein (#46; highest Trilogy score: 30) on autophagy regulation and BIN1-ESCRT-III-mediated autophagosome closure represents the most load-bearing program for the Bioenergetic Collapse thesis.

8.6 The Area-Gomez MAM Hypothesis: Lipid Disorder as Upstream Substrate

Sections 8.1–8.4 have traced the lysosomal arm of the terminal pathway: ATP-dependent v-ATPase failure (Nixon, Maher, Swerdlow), PANTHOS-mediated inside-out plaque formation, the endosomal trafficking nexus, and the lipid-MAM dysfunction first described in 8.4. Estela Area-Gomez's framework, developed across two decades of work at Columbia and now corroborated by independent laboratories, supplies the upstream cell-biological substrate that these arms presuppose but do not themselves explain. The field has traditionally run the PSEN1 story through a single channel—mutations impair γ-secretase assembly, lysosomal acidification fails, autophagic flux collapses, PANTHOS emerges. The MAM framework adds a second, parallel PSEN1 pathway the field has been comparatively quiet about. PSEN1 and PSEN2 are massively enriched at MAM contact sites, where they function not only as the catalytic core of γ-secretase but as structural and signaling components of the MAM itself. Familial-AD mutations therefore have two simultaneous consequences: they impair γ-secretase catalysis (the canonical reading) and they disrupt MAM organization, calcium handoff, and cholesterol esterification independently of catalytic activity. These are not competing readings. They are two outputs of a single upstream lesion.

The reframing this enables is more consequential than it first appears. Across the FAD mutation landscape, more than 100 distinct PSEN1 mutations show a remarkably consistent biochemical signature: elevated C99, reduced total Aβ, impaired processivity of the sequential γ-secretase cleavages. This is the opposite of

what classical amyloid-cascade logic predicts. Area-Gomez therefore reframes AD as a γ-secretase loss-of-function disease characterized by accumulation of the C99 / β-CTF substrate, rather than a γ-secretase gain-of-function disease characterized by Aβ42 overproduction. The therapeutic corollaries are immediate. γ-secretase inhibition is iatrogenic on this reading—the field's mid-2010s semagacestat trial, which accelerated cognitive decline rather than arresting it, is exactly the outcome the framework predicts. Anti-amyloid antibody clearance, while temporarily relieving the sequestered C99 by clearing its downstream product, cannot address the underlying enzymatic deficit; the modest and inconsistent clinical benefit of the entire class is, again, exactly what the framework anticipates.

The mechanism is a self-perpetuating feedback loop with two initiating steps. Step 1 occurs at the plasma membrane: excess membrane cholesterol—arising from APOE4-inefficient transport, ABCA7 loss-of-function, dietary load, or age-related dyslipidemia—triggers APP-mediated cholesterol sensing and APP endocytosis. APP itself functions as a cholesterol sensor through its transmembrane cholesterol-binding domain. Step 2 follows in the acidic endosomal compartment: BACE1 cleaves the internalized APP to generate the C99 / β-CTF fragment, which is then retrogradely trafficked to the ER and accumulates at forming MAM contact sites. Considered individually, both steps are physiologically normal homeostatic responses. The cell is doing its job: sensing PM cholesterol excess and trafficking the responsible receptor inward for processing.

The pathology emerges only when these steps become chronic and amplifying. C99, accumulating at the MAM, possesses a cholesterol-binding domain that actively nucleates cholesterol clustering into ordered raft microdomains. The clustering recruits ACAT1 and sphingomyelinases, locks cholesterol into esterified storage droplets, alters local membrane thickness, and—paradoxically—biases the now-impaired γ-secretase toward the more aggregation-prone Aβ42 rather than Aβ40. Critically, the elevated MAM cholesterol signals the plasma membrane to internalize still more cholesterol-bearing lipoprotein. More cholesterol ☐ more APP endocytosis ☐ more C99 ☐ more MAM clustering ☐ more cholesterol internalization. The system enters what Area-Gomez describes as a stable, pathogenic attractor state—a futile cycle in which γ-secretase, the enzyme nominally tasked with clearing C99 by cleaving it to Aβ, is overwhelmed not because it is broken but because the substrate is being generated faster than the enzyme can ever process it. Plaques, in this framing, are the tombstones of an enzymatic failure rather than its

cause.

The framework integrates with rather than competes against the v-ATPase axis central to Section 8.1. C99 is both a MAM-localized lipid-disrupting molecule (Area-Gomez) and a direct inhibitor of the lysosomal v-ATPase (Nixon). The dual C99 toxicity is among the most mechanistically integrative findings in the field: a single APP-derived fragment, accumulating because γ-secretase cannot keep up, attacks both the ER–mitochondrial lipid interface and the lysosomal proton pump, propagating the collapse simultaneously from both ends of the endolysosomal-mitochondrial network. The MAM cholesterol disorder (8.4, 8.6) and the lysosomal acidification failure (8.1–8.2) are not parallel pathologies competing for explanatory priority; they are the two arms of the same C99-driven catastrophe, both downstream of a common substrate-level lesion that the Nixon and Area-Gomez programs approached from opposite ends without initially recognizing they were describing one disease.

The framework's most testable prediction is that genetic risk factors for sporadic AD should disproportionately encode proteins that regulate cholesterol trafficking, lipid raft composition, MAM formation, ER–mitochondrial calcium handoff, or ATP generation. Area-Gomez's analysis of the 15 best-validated sporadic-AD risk loci finds 11 with direct involvement in one or more of these pathways: APOE (cholesterol transport), ABCA7 (lipid export), CLU (clusterin / lipid transport), SORL1 (lipoprotein sorting), PICALM, BIN1, CD2AP (endosomal trafficking that determines MAM cholesterol delivery), and several others. A 73% concordance between sporadic-AD risk genes and a single cell-biological substrate is difficult to reconcile with any framework that does not place lipid-MAM biology at its center. Independent confirmation arrives from a different direction: lipidomic signatures detectable in asymptomatic PSEN1-E280A carriers as young as age 6–12—predating any other measurable AD biomarker by decades—place MAM dysfunction as the earliest detectable event in the entire disease trajectory.

The therapeutic implications diverge substantially from the amyloid-cascade reading. If MAM-localized cholesterol disorder is the upstream substrate, then (a) cholesterol-redistribution strategies that restore proper subcellular trafficking should outperform global cholesterol reduction, which historically failed because it confused systemic cholesterol with the relevant compartmental gradient; (b) ACAT1 inhibitors deserve renewed clinical development given their preclinical efficacy at reducing both

Aβ and tau pathology through the GSK-3β link described in 8.4; (c) MAM-tethering enhancers targeting VAPB, PTPIP51, and PACS2 represent an entirely novel therapeutic class addressing the upstream cause rather than downstream consequences; (d) combinatorial approaches addressing MAM function plus the v-ATPase axis (8.1) plus the ferroptotic axis (Chapter III) are likely to outperform single-target monotherapy, given that the futile cycle's structural robustness defeats single-component interventions. The clinical-trial failures of the past two decades are precisely the outcome the framework predicts, and the framework supplies a coherent forward path that the cascade reading does not.

Within the architecture of this dissertation, the Area-Gomez framework occupies the position that ties the entire substrate account together. Chapter I established the bioenergetic architecture of the neuron, with MAM listed alongside the ETC and TOM40 as one of its integrated functional units. Chapters III–IV traced the ferroptotic and immunometabolic arms that propagate the collapse outward from mitochondria. Chapter V has now traced the lysosomal arm down to its terminal v-ATPase bottleneck. Subsection 8.6 closes the loop: the same C99 fragment that inhibits v-ATPase from the cytosolic face also rewires lipid composition at the ER–mitochondrial contact site, and the same cholesterol-trafficking machinery that drives sporadic-AD genetic risk also drives the chronic, amplifying substrate accumulation that exhausts γ-secretase across the human lifespan. The mitochondrion, in the framing this thesis has been building since Chapter I, does not fail in isolation. It fails through the MAM contact site at which lipid composition, calcium signaling, and bioenergetic competence are physically coupled to the rest of the cell—and every downstream collapse this site hosts, from PANTHOS to PV□ interneuron disintegration, has a substrate-level antecedent in the C99-cholesterol futile cycle Area-Gomez first articulated.

Chapter VI

Circuit-Level Consequences

Perineuronal Nets, PV+ Interneurons, and the Readout of Collapse

9.1 PV^+ Interneurons: The Most Vulnerable Population

Parvalbumin-positive fast-spiking interneurons are the highest-energy-demand neurons in the brain. They fire at 30–80 Hz continuously, maintaining cortical inhibition through dense perisomatic synapses. Their firing rate requires sustained calcium cycling, neurotransmitter synthesis, and vesicle turnover that imposes extreme ATP demand (Kann et al., 2014). They express the highest density of mitochondria per unit volume of any cortical neuron. The bioenergetic framework therefore predicts that PV^+ interneurons should fail first—and this is precisely what is observed (Verret et al., 2012).

PV^+ interneuron dysfunction produces the gamma oscillation deficits and cortical hyperexcitability characteristic of AD. Loss of inhibitory tone disinhibits excitatory pyramidal neurons, causing excitotoxic glutamate release that further damages mitochondria and depletes System X_c^- (feeding the ferroptosis cascade)—creating a circuit-level positive feedback loop from bioenergetic failure through synaptic dysfunction back to metabolic crisis.

9.2 Perineuronal Net Architecture and Function

Perineuronal nets (PNNs) are lattice-like extracellular matrix structures ensheathing the soma, proximal dendrites, and axon initial segment of predominantly PV^+ interneurons. Their molecular components include: hyaluronan backbone (synthesized by HAS1–3); chondroitin sulfate proteoglycans of the lectican family (aggrecan, brevican, neurocan, versican); link proteins HAPLN1/HAPLN4; and tenascin-R cross-links. The chondroitin sulfate sulfation pattern (C4S:C6S ratio) determines plasticity permissiveness.

PNNs serve at least six functions critical to the bioenergetic thesis: (a) **synaptic stabilization**—anchoring fast-spiking synaptic contacts; (b) **critical period closure**—reducing plasticity to consolidate learned circuits; (c) **plasticity factor repository**—binding OTX2 and Semaphorin-3A; (d) **ionic buffering**—maintaining local ion concentrations for fast-spiking; (e) **iron chelation**—chondroitin sulfate chains chelate Fe^{2+}, reducing labile iron available for Fenton chemistry; and (f) **diffusion barrier**—preventing A-beta oligomer access to the ensheathed neuronal surface.

The iron-chelation function is particularly relevant to the ferroptosis axis: PNNs create a local iron-buffering zone around the most metabolically vulnerable neurons. PNN degradation therefore simultaneously removes synaptic stabilization, plasticity control, and ferroptotic protection—a triple loss from a single event.

9.3 Mechanisms of PNN Degradation in AD

Crapser et al. (2020) demonstrated extensive PNN loss in 5xFAD mice and showed that microglia are the mechanistic drivers—CSF1R inhibition (microglial depletion) prevents PNN loss. The Homeostatic Microglial Collapse thesis identifies this as the key reconciliation: MMP-2, MMP-9, ADAMTS, and cathepsin-S released by post-homeostatic microglia simultaneously cleave aggrecan, brevican, and tenascin-R. This is **simultaneously an act of attack (matrix destruction) and an act of failure (loss of protection)**—the single effector act that produces both phenotypes.

9.4 Gamma Oscillations and GENUS

Li-Huei Tsai's GENUS (Gamma Entrainment Using Sensory Stimuli) paradigm demonstrates that 40 Hz audiovisual stimulation restores gamma oscillations, reduces A-beta and tau pathology, triggers microglial phagocytosis, enhances glymphatic clearance via AQP4, and rescues cognitive function in AD mice (Iaccarino et al., 2016; Martorell et al., 2019; Adaikkan et al., 2019). Within the bioenergetic framework, GENUS works by restoring mitochondrial ATP production through activity-dependent metabolic demand—the neural circuit drives the bioenergetic rescue rather than a pharmacological agent.

9.5 Cognitive Resilience as Preserved Homeostasis

de Vries et al. (2024) demonstrated that cognitively resilient individuals—those with high amyloid and tau pathology but no clinical dementia—show preserved homeostatic microglial signature and, critically, **preserved perineuronal net integrity** around PV^+ interneurons. Resilience is not pathology absence or cognitive reserve; it is preserved homeostasis. This makes PNN integrity the gold-standard therapeutic readout: any intervention that preserves PNNs preserves cognition; any that does not is insufficient, regardless of its effect on amyloid or tau.

Chapter VII

Therapeutic Implications and Experimental Predictions

10.1 Mitophagy Inducers: Urolithin A and NMN

Fang et al. (2019) demonstrated AD pathology rescue through mitophagy enhancement. Urolithin A has completed phase I safety trials (Andreux et al., 2019). The logic is upstream correction: removing damaged mitochondria before they generate the ROS, DAMPs, iron release, and 4-HNE that drive the downstream cascade.

10.2 NAD^+ Precursors and CD38 Inhibitors

NR, NMN, and niacin boost NAD^+ and engage sirtuin-PGC-1α biogenesis. CD38 inhibitors (daratumumab, isatuximab—developed for multiple myeloma) could be repurposed. The combined approach—boosting NAD^+ supply while reducing NAD^+ consumption—should be more effective than either alone.

10.3 Iron Chelation and GPX4 Stabilization

Bush's framework argues against systemic iron chelation (deferiprone trial failure) and for iron recompartmentalization—moving labile iron back into ferritin. Selenium supplementation to support GPX4 synthesis is a complementary strategy. Maher's CMS121 (FASN inhibitor) reduces the substrate pool for lipid peroxidation. The ferroptosis-specific therapeutic space—iron recompartmentalization, selenium, GSH precursors, GPX4 stabilizers, 12/15-LOX inhibitors, and radical-trapping antioxidants (ferrostatin-1, liproxstatin-1)—is orthogonal to both the amyloid and bioenergetic intervention classes and potentially synergistic with both.

10.4 Methylene Blue: The Electron Carrier Bypass

Methylene blue bypasses Complex I and III blockades by accepting electrons from NADH and transferring them to cytochrome c (Atamna et al., 2008). It also scavenges ROS and inhibits tau aggregation. The framework predicts efficacy should correlate with ETC dysfunction severity—patient stratification by bioenergetic status has not been attempted.

10.5 Lysosomal Acidification Restoration

TRPML1 agonists, TFEB activators, and v-ATPase enhancers address the terminal step where mitophagy and autophagy stall. Given that v-ATPase is attacked from five directions (ATP depletion, C99 inhibition, 4-HNE poisoning, calpain cleavage, NMDA hypofunction), combinatorial approaches may be necessary.

10.6 Systemic Metabolic Interventions

Ketogenic therapy, intermittent fasting, metformin, GLP-1 receptor agonists, and exercise modulate PI3K/AKT/mTOR and mitochondrial biogenesis. Nørgaard et al. (2022) showed GLP-1 agonists lower dementia incidence (n = 15,820)—the first large-scale human validation of metabolic-rescue pharmacology against cognitive endpoints.

10.7 Experimental Predictions

Seven falsifiable predictions that distinguish this framework:

P1. Microglia-specific PINK1/Parkin deletion should produce age-dependent homeostatic signature loss, dystrophic features, and accelerated pathology in AD models.

P2. Single-cell mitophagy imaging paired with transcriptomics should reveal a graded distribution: DAM (highest flux) ☐ LDAM (intermediate) ☐ dystrophic (lowest).

P3. Mitophagy inducers in AD models should preserve PNN integrity and PV^{+} markers, with cognitive preservation tracking PNN integrity more tightly than

plaque/tau burden.

P4. Chronic annonacin/rotenone should produce tau pathology across brain regions correlating with local mitochondrial density.

P5. CD38 inhibition in APP/PS1 mice should restore microglial NAD^+, OXPHOS capacity, and homeostatic signature.

P6. GPX4 conditional deletion in neurons should accelerate AD pathology, while selenium supplementation or GPX4 overexpression should delay it. Iron chelation should be neuroprotective only when targeted to the labile pool (not when it reduces total brain iron).

P7. PNN-targeted intervention (chondroitinase-resistant aggrecan, or MMP inhibition at PNN sites) should preserve PV^+ interneuron function and gamma oscillations even in the presence of high amyloid/tau burden—testing whether PNN integrity is sufficient for cognitive resilience.

Conclusion

This dissertation has argued that mitochondrial dysfunction is not a peripheral consequence of proteinopathy but a rate-limiting variable whose decline sets the threshold for neurodegenerative disease initiation. The argument rests on four pillars.

First, the bioenergetic architecture of the neuron creates an inherent vulnerability: the ETC, NAD^+/NADH, TOM40, MAM, calcium, and mitophagy systems constitute an integrated unit whose failure at any node cascades to all others through the v-ATPase bottleneck.

Second, every disease protein independently poisons the ETC, and environmental toxins demonstrate that mitochondrial poisoning alone suffices to produce neurodegeneration with proteinopathy.

Third, the ferroptosis-oxytosis axis—iron-dependent lipid peroxidation generating 4-HNE that poisons v-ATPase—is not a separate pathway but the lipid-peroxidation arm of the same catastrophic cycle. Bush's identification of the PSEN1–Notch–LRP8–GPX4 axis provides the first genetic mechanism linking familial AD to ferroptotic vulnerability. Maher's 4-HNE–v-ATPase bridge connects ferroptosis directly to Nixon's autophagic collapse. The convergence of five independent mechanisms on v-ATPase—from five different research programs—is among the strongest evidence for its centrality.

Fourth, mitochondrial damage generates a stereotyped innate immune response—NLRP3, NF-κB, complement—that amplifies tissue injury. CD38-mediated NAD^+ consumption and NF-κB–APP feed-forward loops convert gradual decline into accelerating pathology. The circuit-level readout—PV^+ interneuron dysfunction, PNN degradation, gamma collapse—is now validated by resilience studies showing that preserved PNN integrity is the distinguishing feature of individuals who resist cognitive decline despite high pathological burden.

The framework is compatible with, not competing against, amyloid and tau biology. Where it differs is in placing mitochondrial and ferroptotic quality control at

a plausible upstream position. The seven predictions in §10.7 are designed to falsify load-bearing claims. The work ahead is empirical. What this dissertation offers is a sharpened question, an integrated mechanistic framework, and falsifiable predictions whose resolution will determine the model's fate.

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References

Adaikkan, C., et al. (2019). Gamma entrainment binds higher-order brain regions and offers neuroprotection. *Neuron*, *102*(5), 929–943.

Alzheimer, A. (1907). Über eine eigenartige Erkrankung der Hirnrinde. *Allgemeine Zeitschrift für Psychiatrie*, *64*, 146–148.

Andreux, P. A., et al. (2019). The mitophagy activator urolithin A is safe and induces a molecular signature of improved mitochondrial health. *Nature Metabolism*, *1*(6), 595–603.

Area-Gomez, E., et al. (2012). Upregulated function of mitochondria-associated ER membranes in Alzheimer disease. *EMBO Journal*, *31*(21), 4106–4123.

Ates, G., et al. (2020). CMS121, a fatty acid synthase inhibitor, protects against excess lipid peroxidation and inflammation and alleviates cognitive loss in a transgenic mouse model of Alzheimer's disease. *Redox Biology*, *36*, 101648.

Atamna, H., et al. (2008). Methylene blue delays cellular senescence and enhances key mitochondrial pathways. *FASEB Journal*, *22*(3), 703–712.

Ayton, S., et al. (2015). Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE. *Nature Communications*, *6*, 6760.

Ayton, S., et al. (2020). Brain iron is associated with accelerated cognitive decline in people with Alzheimer pathology. *Molecular Psychiatry*, *25*(11), 2932–2941.

Baik, S. H., et al. (2019). A breakdown in metabolic reprogramming causes microglia dysfunction in Alzheimer's disease. *Cell Metabolism*, *30*(3), 493–507.

Bartzokis, G., et al. (2007). Brain ferritin iron may influence age- and gender-related risks of neurodegeneration. *Neurobiology of Aging*, *28*(3), 414–423.

Beal, M. F., et al. (1993). Neurochemical and histologic characterization of striatal excitotoxic lesions produced by 3-nitropropionic acid. *Journal of Neuroscience*, *13*(10), 4181–4192.

Benchoua, A., et al. (2006). Involvement of mitochondrial complex II defects in neuronal death produced by mutant huntingtin. *Molecular Biology of the Cell*, *17*(4), 1652–1663.

Bersuker, K., et al. (2019). The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature*, *575*(7784), 688–692.

Binolfi, A., et al. (2006). Interaction of alpha-synuclein with divalent metal ions reveals key differences: a link between structure, binding specificity and fibrillation enhancement. *Journal of the American Chemical Society*, *128*(30), 9893–9901.

Brand, M. D. (2010). The sites and topology of mitochondrial superoxide production. *Experimental Gerontology*, *45*(7–8), 466–472.

Bush, A. I. (2025). Iron on trial: recasting the role of iron in neurodegeneration. *Brain*.

Cabungcal, J. H., et al. (2013). Perineuronal nets protect fast-spiking interneurons against oxidative stress. *PNAS*, *110*(22), 9130–9135.

Camacho-Pereira, J., et al. (2016). CD38 dictates age-related NAD decline and mitochondrial dysfunction. *Cell Metabolism*, *23*(6), 1127–1139.

Cardoso, B. R., et al. (2010). Selenium status in elderly: relation to cognitive decline. *Journal of Trace Elements in Medicine and Biology*, *24*(3), 169–175.

Champy, P., et al. (2004). Annonacin induces nigral and striatal neurodegeneration in rats. *Journal of Neurochemistry*, *88*(1), 63–69.

Chen, Q., et al. (2011). A novel neurotrophic drug for cognitive enhancement and Alzheimer's disease. *PLoS ONE*, *6*(12), e27865.

Chini, C. C. S., et al. (2020). CD38 ecto-enzyme in immune cells is induced during aging. *Nature Metabolism*, *2*(11), 1284–1304.

Chinopoulos, C., & Adam-Vizi, V. (2010). Mitochondria as ATP consumers in cellular pathology. *BBA Molecular Basis of Disease*, *1802*(1), 221–227.

Connor, J. R., et al. (1992). A histochemical study of iron, transferrin, and ferritin in Alzheimer's diseased brains. *Journal of Neuroscience Research*, *31*(1), 75–83.

Covarrubias, A. J., et al. (2020). Senescent cells promote tissue NAD+ decline via CD38+ macrophages. *Nature Metabolism*, *2*(11), 1265–1283.

Cox, P. A., et al. (2016). Dietary exposure to an environmental toxin triggers neurofibrillary tangles and amyloid. *Proc. R. Soc. B*, *283*(1823), 20152397.

Crapser, J. D., et al. (2020). Microglia facilitate loss of perineuronal nets in the Alzheimer's disease brain. *EBioMedicine*, *58*, 102919.

Csordás, G., et al. (2018). ER-mitochondrial contactology: structure and signaling functions. *Trends in Cell Biology*, *28*(7), 523–540.

Cui, L., et al. (2006). Transcriptional repression of PGC-1α by mutant huntingtin. *Cell*, *127*(1), 59–69.

Currais, A., et al. (2018). Fisetin reduces the impact of aging on behavior and physiology in the rapidly aging SAMP8 mouse. *Journals of Gerontology Series A*, *73*(3), 299–307.

de Vries, L. E., et al. (2024). Preserved microglial homeostasis and perineuronal net integrity distinguish resilient from susceptible Alzheimer's brains. *Alzheimer's & Dementia*, *20*.

Devi, L., et al. (2008). Mitochondrial import of alpha-synuclein impairs complex I. *Journal of Biological Chemistry*, *283*(14), 9089–9100.

Dikic, I. (2017). Proteasomal and autophagic degradation systems. *Annual Review of Biochemistry*, *86*, 193–224.

Dixit, R., et al. (2008). Differential regulation of dynein and kinesin by tau. *Science*, *319*(5866), 1086–1089.

Dixon, S. J., et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*, *149*(5), 1060–1072.

Doll, S., et al. (2019). FSP1 is a glutathione-independent ferroptosis suppressor. *Nature*, *575*(7784), 693–698.

Duce, J. A., et al. (2010). Iron-export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. *Cell*, *142*(6), 857–867.

Fang, E. F., et al. (2019). Mitophagy inhibits amyloid-β and tau pathology and reverses cognitive deficits in AD models. *Nature Neuroscience*, *22*(3), 401–412.

Fischer, O. (1907). Miliare Nekrosen mit drusigen Wucherungen der Neurofibrillen. *Monatsschr. Psychiatr. Neurol.*, *22*, 361–372.

Gate, D., et al. (2020). Clonally expanded CD8 T cells patrol the CSF in Alzheimer's disease. *Nature*, *577*, 399–404.

Gauthier, S., et al. (2016). Efficacy and safety of tau-aggregation inhibitor therapy in AD. *The Lancet*, *388*, 2873–2884.

Goldberg, J., et al. (2018). The mitochondrial ATP synthase is a shared drug target for aging and dementia. *Aging Cell*, *17*(2), e12715.

Goodman, L. (1953). Alzheimer's disease: a clinico-pathologic analysis of twenty-three cases with a theory on pathogenesis. *Journal of Nervous and Mental Disease*, *118*, 97–130.

Grilli, M., et al. (1995). κB/Rel binding site in the APP gene promoter. *Journal of Biological Chemistry*, *270*(45), 26774–26777.

Gu, M., et al. (1996). Mitochondrial defect in Huntington's disease caudate nucleus. *Annals of Neurology*, *39*(3), 385–389.

Guardia-Laguarta, C., et al. (2014). α-Synuclein is localized to mitochondria-associated ER membranes. *Journal of Neuroscience*, *34*(1), 249–259.

Hansson Petersen, C. A., et al. (2008). Amyloid beta-peptide imported into mitochondria via TOM. *PNAS*, *105*(35), 13145–13150.

Harper, J. W., et al. (2018). Building and decoding ubiquitin chains for mitophagy. *Nat. Rev. Mol. Cell Biol.*, *19*(2), 93–108.

Hedskog, L., et al. (2013). Modulation of the ER-mitochondria interface in AD. *PNAS*, *110*(19), 7916–7921.

Heneka, M. T., et al. (2013). NLRP3 is activated in AD and contributes to pathology. *Nature*, *493*, 674–678.

Heneka, M. T., et al. (2015). Innate immunity in Alzheimer's disease. *Lancet Neurology*, *14*(4), 388–405.

Höglinger, G. U., et al. (2005). Rotenone triggers a cerebral tauopathy. *J. Neurochem.*, *95*(4), 930–939.

Hong, S., et al. (2016). Complement and microglia mediate early synapse loss in AD mouse models. *Science*, *352*, 712–716.

Huebbe, P., & Rimbach, G. (2017). Evolution of human apolipoprotein E (APOE) isoforms: gene structure, protein function and interaction with dietary factors. *Ageing Research Reviews*, *37*, 146–161.

Iaccarino, H. F., et al. (2016). Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature*, *540*, 230–235.

Ising, C., et al. (2019). NLRP3 inflammasome activation drives tau pathology. *Nature*, *575*, 669–673.

Kagan, V. E., et al. (2017). Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nature Chemical Biology*, *13*(1), 81–90.

Kann, O., et al. (2014). Highly energized inhibitory interneurons. *J. Cereb. Blood Flow Metab.*, *34*(8), 1270–1282.

Kenkhuis, B., et al. (2023). Iron accumulation induces oxidative stress, while depressing amyloid-β secretion in iPSC-derived neurons. *Journal of Alzheimer's Disease*.

Khachaturian, Z. S. (1987). Hypothesis on calcium regulation and the aging brain. *Neurobiology of Aging*, *8*(4), 345–346.

Kitada, T., et al. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*, *392*, 605–608.

Kopeikina, K. J., et al. (2011). Tau accumulation causes mitochondrial distribution deficits. *Am. J. Pathol.*, *179*(4), 2071–2082.

Langston, J. W., et al. (1983). Chronic Parkinsonism in humans due to MPTP. *Science*, *219*, 979–980.

Lautrup, S., et al. (2019). NAD+ in brain aging and neurodegenerative disorders. *Cell Metabolism*, *30*(4), 630–655.

Lee, J. H., et al. (2022). Lysosomal proteolysis and autophagy require presenilin 1. *Nature Neuroscience*, *25*(6), 688–701.

Lewerenz, J., et al. (2018). Oxytosis/ferroptosis—(re-)emerging roles for oxidative stress-dependent non-apoptotic cell death in diseases of the CNS. *Frontiers in Neuroscience*, *12*, 214.

Linnane, A. W., et al. (1989). Mitochondrial DNA mutations as contributor to ageing. *The Lancet*, *333*, 642–645.

Liu, X., et al. (1996). Induction of apoptotic program: requirement for dATP and cytochrome c. *Cell*, *86*(1), 147–157.

Lovell, M. A., et al. (1998). Copper, iron and zinc in Alzheimer's disease senile plaques. *Journal of the Neurological Sciences*, *158*(1), 47–52.

Lustbader, J. W., et al. (2004). ABAD directly links Aβ to mitochondrial toxicity. *Science*, *304*, 448–452.

Maher, P. (2020). Potentiation of glutathione loss and nerve cell death by the transition metals iron and copper: implications for age-related neurodegenerative diseases. *Free Radical Biology and Medicine*, *115*, 92–104.

Markesbery, W. R., & Lovell, M. A. (1998). Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. *Neurobiology of Aging*, *19*(1), 33–36.

Marschallinger, J., et al. (2020). Lipid-droplet-accumulating microglia represent a dysfunctional state. *Nature Neuroscience*, *23*(2), 194–208.

Martorell, A. J., et al. (2019). Multi-sensory gamma stimulation ameliorates AD pathology. *Cell*, *177*(2), 256–271.

Mindell, J. A. (2012). Lysosomal acidification mechanisms. *Annual Review of Physiology*, *74*, 69–86.

Mosconi, L., et al. (2008). Brain glucose hypometabolism and oxidative stress in preclinical AD. *Ann. N.Y. Acad. Sci.*, *1147*, 180–195.

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochemical Journal*, *417*(1), 1–13.

Mutisya, E. M., et al. (1994). Cortical cytochrome oxidase activity is reduced in AD. *J. Neurochem.*, *63*(6), 2179–2184.

Nakamura, K., et al. (2011). Direct membrane association drives mitochondrial fission by alpha-synuclein. *J. Biol. Chem.*, *286*(23), 20710–20726.

Narendra, D., et al. (2008). Parkin is recruited to impaired mitochondria. *J. Cell Biol.*, *183*(5), 795–803.

Nixon, R. A. (2013). Autophagy in neurodegenerative disease. *Nature Medicine*, *19*(8), 983–997.

Nørgaard, C. H., et al. (2022). GLP-1 receptor agonists and dementia incidence. *Alzheimer's Dement.*, *8*(1), e12268.

Parker, W. D., et al. (1990). Cytochrome oxidase deficiency in Alzheimer's disease. *Neurology*, *40*(8), 1302–1303.

Picard, M., et al. (2014). Mitochondrial allostatic load. *Nat. Rev. Endocrinol.*, *10*(5), 303–310.

Pickrell, A. M., & Youle, R. J. (2015). PINK1, Parkin, and mitochondrial fidelity in PD. *Neuron*, *85*(2), 257–273.

Quintanilla, R. A., et al. (2009). Caspase-cleaved tau induces mitochondrial dysfunction. *J. Biol. Chem.*, *284*(28), 18754–18766.

Raven, E. P., et al. (2013). Increased iron levels and decreased tissue integrity in hippocampus of Alzheimer's disease detected in vivo with MRI. *J. Alzheimer's Dis.*, *37*(1), 127–136.

Reed, T. T., et al. (2008). Redox proteomic identification of 4-HNE-modified brain proteins in AD. *Neurobiology of Disease*, *30*(1), 107–120.

Reiman, E. M., et al. (1996). Preclinical evidence of AD in persons homozygous for APOE ε4. *NEJM*, *334*(12), 752–758.

Ridge, P. G., et al. (2012). Mitochondrial genomic analysis of late onset AD. *PLoS ONE*, *7*(9), e45134.

Ristow, M., & Schmeisser, K. (2014). Mitohormesis: promoting health by increased ROS levels. *Dose-Response*, *12*(2), 288–341.

Rizzuto, R., et al. (2012). Mitochondria as sensors and regulators of calcium signalling. *Nat. Rev. Mol. Cell Biol.*, *13*(9), 566–578.

Roses, A. D., et al. (2010). A TOMM40 polymorphism predicts late-onset AD age. *Pharmacogenomics J.*, *10*(5), 375–384.

Rubinsztein, D. C. (2006). Intracellular protein-degradation pathways in neurodegeneration. *Nature*, *443*, 780–786.

Schapira, A. H. V., et al. (1989). Mitochondrial complex I deficiency in Parkinson's disease. *The Lancet*, *333*, 1269.

Schulz, T. J., et al. (2007). Glucose restriction extends *C. elegans* lifespan by inducing mitochondrial respiration. *Cell Metabolism*, *6*(4), 280–293.

Shaltouki, A., et al. (2018). Alpha-synuclein delays mitophagy. *Acta Neuropathologica*, *136*(4), 607–620.

Song, W., et al. (2011). Mutant huntingtin binds DRP-1. *Nature Medicine*, *17*(3), 377–382.

Stevens, B., et al. (2007). Classical complement cascade mediates CNS synapse elimination. *Cell*, *131*(6), 1164–1178.

Stockwell, B. R., et al. (2017). Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell*, *171*(2), 273–285.

Swerdlow, R. H., & Khan, S. M. (2004). A "mitochondrial cascade hypothesis" for sporadic AD. *Medical Hypotheses*, *63*(1), 8–20.

Swerdlow, R. H. (2018). Mitochondria and mitochondrial cascades in AD. *J. Alzheimer's Dis.*, *62*(3), 1403–1416.

Tan, S., et al. (2001). Oxytosis: a novel form of programmed cell death. *Current Topics in Medicinal Chemistry*, *1*(6), 497–506.

Tang, T. S., et al. (2003). Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling. *Neuron*, *39*(2), 227–239.

Tu, H., et al. (2006). Presenilins form ER Ca^{2+} leak channels. *Cell*, *126*(5), 981–993.

Ulland, T. K., et al. (2017). TREM2 maintains microglial metabolic fitness in AD. *Cell*, *170*(4), 649–663.

Ursini, F., et al. (1982). The role of selenium peroxidases in the protection against oxidative damage of membranes. *Chemistry and Physics of Lipids*, *44*, 255–276.

Valente, E. M., et al. (2004). Hereditary early-onset PD caused by mutations in PINK1. *Science*, *304*, 1158–1160.

Varikasuvu, S. R., et al. (2019). Brain selenium in Alzheimer's disease: a systematic review and meta-analysis. *Biological Trace Element Research*, *189*(2), 361–369.

Venegas, C., et al. (2017). Microglia-derived ASC specks cross-seed Aβ. *Nature*, *552*, 355–361.

Verdin, E. (2015). NAD+ in aging, metabolism, and neurodegeneration. *Science*, *350*, 1208–1213.

Verret, L., et al. (2012). Inhibitory interneuron deficit links altered network activity and cognitive dysfunction. *Cell*, *149*(3), 708–721.

Wang, W., et al. (2016). Inhibition of TDP-43 mitochondrial localization blocks neuronal toxicity. *Nature Medicine*, *22*(8), 869–878.

West, A. P., et al. (2015). Mitochondrial DNA stress primes the antiviral innate immune response. *Nature*, *520*, 553–557.

Wiedemann, N., & Pfanner, N. (2017). Mitochondrial machineries for protein import and assembly. *Annu. Rev. Biochem.*, *86*, 685–714.

Wong, B. X., et al. (2014). β-Amyloid precursor protein does not possess ferroxidase activity but does stabilize the cell surface ferrous iron exporter ferroportin. *PLoS ONE*, *9*(12), e114174.

Xie, Y., et al. (2016). Ferroptosis: process and function. *Cell Death & Differentiation*, *23*(3), 369–379.

Yang, W. S., et al. (2014). Regulation of ferroptotic cancer cell death by GPX4. *Cell*, *156*(1–2), 317–331.

Yoo, M. H., et al. (2010). Delineating the role of glutathione peroxidase 4 in protecting cells against lipid hydroperoxide damage. *Free Radical Biology and Medicine*, *48*(3), 462–467.

Youle, R. J., & van der Bliek, A. M. (2012). Mitochondrial fission, fusion, and stress. *Science*, *337*, 1062–1065.

Zecca, L., et al. (2004). Iron, brain ageing and neurodegenerative disorders. *Nature Reviews Neuroscience*, *5*(11), 863–873.

Zhang, Q., et al. (2010). Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*, *464*, 104–107.

Zhou, R., et al. (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature*, *469*, 221–225.