
The Oxidised Eraser

*The Speck, the Thiol and the Inhibitor — Whether Amyloid- β and the Oxidative
Burst It Licenses Disable One Phosphatase by Three Routes at Once, and What
Each of Those Routes Actually Touches*

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Benjamin Aaron Gustafsson

AdultCognitiveDisease.com

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*Three routes were said to converge on one phosphatase. They converge on a noun. Each acts on a different holoenzyme, and
the only one with human evidence has neither an oxidative trigger nor the tau-directed target.*

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Abstract

The companion volume *The Four Locks* asked whether the four described ways of switching off protein phosphatase 2A inside the cortical neuron converge, and found that they converge on a target and not on a trigger. Oxidative stress, the field's default candidate for the shared cause, drew fewer arrows than assumed: it reaches calpain through calcium rather than through aldehyde chemistry, it does not reach asparaginyl endopeptidase, whose maturation it deranges — but it does reach the phosphatase directly, through a vicinal thiol pair on the catalytic subunit whose disulfide formation is reversible with dithiothreitol. This volume takes that surviving arm and asks whether it is the trigger after all: whether amyloid- β , acting through the ASC speck and the oxidative burst it licenses, disables the phosphatase by three chemically distinct routes at once — covalent oxidation of the catalytic subunit's own thiols, relocation of the methylesterase PME-1 and demethylation of leucine 309, and induction of the inhibitor CIP2A.

The general proposition is not ours to claim, and we say so before anything else. That oxidative stress modulates this phosphatase system, endogenous inhibitors and all, was reviewed in *Pharmacology & Therapeutics* in 2019, in an article that covers CIP2A and SET explicitly and proposes CIP2A/SET antagonists as therapy. Its own verdict — that free-radical-mediated attenuation of phosphatase activity is an emerging theme, but that in many instances a dichotomy exists — is the honest state of the field, and this volume does not improve on it at that altitude. What is unclaimed is narrower: the neuron, the disease, the buffer, the two departures from the nucleus, and one experiment.

The answer to the question as asked is no, and the reason is more specific than thin evidence. The three routes do not act on the same molecule. Oxidation of the vicinal thiol pair acts on the catalytic subunit and is indifferent to which holoenzyme carries it. Loss of the methyl ester at leucine 309 acts on recruitment of the B55 α subunit, which is the subunit that makes this enzyme a tau phosphatase. And CIP2A — the route with by far the strongest human evidence — binds B56 α and B56 γ , on a crystal structure and a mutational analysis, and B56 is not that subunit. Three routes, three molecular objects, one noun. **This is the same subtraction *The Four Locks* performed on SET, and it is forced by the same kind of evidence: a pull-down and a structure, not a failure of replication.**

Each arm is graded, and the grades do not fall where the list predicts. The speck-to-neuron arm is **Weak**: the only direct demonstration that an ASC speck raises reactive oxygen species in anything neuron-like is a single result in SH-SY5Y cells, reported inside a study of ethanol binge-drinking, using specks produced by glia; no study anywhere shows an extracellular speck entering a neuron, and neuronal expression of the sensor is reagent-dependent in a literature where eight anti-NLRP3 antibodies failed formal validation. The disulfide arm is **Moderate and rat-only**, weakened by its own laboratory twice — a survey of vicinal thiol oxidation in healthy rat brain recovered Na,K-ATPase, tubulins, glyceraldehyde-3-phosphate dehydrogenase and protein phosphatase 1, with protein phosphatase 2A absent from the list, and a later survey found these redox states stable across the rat lifespan. S-glutathionylation of the catalytic subunit is not

demonstrated and should not be asserted; the candidate pair can nonetheless be named, because cysteines 266 and 269 are the only vicinal thiols in the protein and the widely repeated claim that cysteines 50 and 55 form a second pair is structurally false. The methylesterase arm has good mechanism in the wrong cell types and one genuinely informative human result: the methylesterase is **increased in abundance** in Alzheimer and progressive supranuclear palsy cortex, with the methylation ratio down about seventy-five per cent — which supports the inflammatory route of the companion volume and not the redox route this one was written to test. The CIP2A arm is **Strong and human**, and its demonstrated upstream is amyloid- β to DNA damage to checkpoint kinase 1.

One correction we owe to our own commissioning brief. That brief recorded the redox bridge to CIP2A as undrawn and instructed that the corpus must not assert it. The instruction was based on abstracts. In the full text of the checkpoint-kinase-1 study the bridge is drawn, in a **primary neuron**: hydrogen peroxide induced checkpoint-kinase-1 activation, CIP2A overexpression, phosphatase suppression and tau hyperphosphorylation, all reversed by inhibiting the kinase. It is the only experiment in this volume containing an oxidant, a neuron and this phosphatase together, and it is better than we expected — subject to a two-hour supraphysiological bolus, no measurement of the DNA lesion, no identified sensor kinase, and two objections from neuronal physiology that this volume raises and the literature has not answered: the checkpoint is a replication-stress checkpoint and a neuron does not replicate, and CIP2A's role in that response is gated on nuclear-envelope breakdown, which a neuron never performs.

Two findings are new and are the reason the volume exists. The first is structural. The methylesterase leaves the nucleus under oxidative stress by CRM1-dependent export with its abundance unchanged; the inhibitor SET leaves the nucleus in Alzheimer tissue because the cut at asparagine 175 bisects the localisation signal that held it there. Two independent regulators of one catalytic subunit, departing one compartment, by unrelated mechanisms, in the same disease. Nobody has put those observations on the same page. What the juxtaposition yields is not a unified mechanism — the mechanisms have nothing in common and only one of them is irreversible — but a redirection: the shared consequence is a *loss in the nucleus*, not a gain in the cytoplasm, and the nuclear half of this lesion has never been measured in a neuron.

The second finding is methodological, and verifying it weakened it before it strengthened it. *The Four Locks* asserted that every human phosphatase-activity measurement was made in buffer containing ten millimolar β -mercaptoethanol, so that any redox component of the deficit was reduced away before it could be counted. We audited the primary methods paper by paper. **The recipe is verified verbatim in two papers from the laboratory concerned — and in the four papers that carry the canonical numbers the buffer is not recoverable from anything we can access.** The claim is therefore a well-motivated prediction about four methods sections, not an established fact, and the companion volume's unconditional version is corrected here. To it we add an independent erasure that depends on no buffer at all: in rat brain, decapitation-induced global ischaemia produces a transient **reductive** shift in protein vicinal thiol redox state. If that holds in human tissue, the manner of death reduces the lesion before the sample is collected. We also enter a negative human study that neither companion volume carries, in which no phosphatase activity differed between Alzheimer and control tissue at all.

What is withdrawn, and what is added. We downgrade *The Four Locks'* ledger entry grading reversible vicinal-thiol inhibition of the phosphatase as **Strong** to **Moderate**, on the two Foley surveys that volume did not carry, and restate its buffer claim as conditional. We correct its report that no genetic association of this pathway has been published: one has been tested across six genes in nearly fourteen hundred subjects and was negative. We correct the claim that two CIP2A-lowering compounds have rescued Alzheimer phenotypes: one has, in 3×Tg-AD mice and primary neurons; the other was tested only in transfected human embryonic kidney cells. And we add CIP2A to a corpus in which it had zero occurrences — with three warnings: its structurally defined target is the wrong holoenzyme for tau, every human measurement of it is a bulk immunoblot never resolved to a cell type in tissue where its own investigators report it higher in astrocytes than neurons, and every transfer from oncology to the neuron must be labelled as a transfer.

The experiment. A redox-shift blot — polyethylene-glycol maleimide or biotin switch — of the phosphatase catalytic subunit from human Alzheimer and control cortex, homogenised without reducing agent and alkylated at the moment of disruption to trap the in vivo thiol state, run beside a reducing-agent-free activity re-assay with dithiothreitol added back. No arm of this literature has performed it in human tissue, the positive controls are already named by the rat surveys, and it is the measurement the buffer problem predicts. It costs a freezer aliquot and an afternoon, and it can falsify the redox arm outright.

I. The List and the Question

A list is not a pathway, and the danger of the list is that the page supplies a premise the evidence has not. Seven items were written one beneath another — the ASC speck, amyloid- β , PME-1, CIP2A, oxidative stress, the disulfide bridge, protein phosphatase 2A — and read downward they compose a sentence: amyloid assembles a speck, the speck licenses an oxidative burst, the burst oxidises a thiol pair, demethylates a leucine and induces an inhibitor, and the phosphatase goes dark. Every arrow in that sentence has been drawn somewhere in the literature. The question this volume exists to answer is whether they have been drawn in the same cell, in the same species, in the same disease, and onto the same molecule.

The companion volume answered a narrower version of this question and got a two-part answer that constrains everything below. *The Four Locks* assembled the four described mechanisms for switching off this phosphatase inside the cortical neuron — demethylation at leucine 309, phosphorylation of a tyrosine, occupancy by an endogenous inhibitor, and proteolysis of that inhibitor at asparagine 175 — and found that three of them converge on a single functional statement, that the catalytic subunit and tau are not brought together, while their upstream afferents converge on nothing at all. One-carbon metabolism, amyloid-driven kinase activity, lysosomal membrane failure and calcium are four separate inputs with four separate time courses. The architecture is a coincidence structure rather than a cascade: an enzyme with an

unusual number of independent regulatory surfaces, sitting in a cell subject to an unusual number of simultaneous insults.

That volume also found one arrow it had not expected to find. Of the several routes by which oxidative stress is supposed to reach this mechanism, most did not survive — it does not activate the protease, whose cysteine oxidation deranges maturation; the aldehydes of lipid peroxidation inhibit calpain rather than activating it; its connection to the inhibitor's transcription is inferred from a promoter element and never tested with an oxidant. But one route survived intact, and it was the least cited of them: reactive species inhibit the phosphatase *directly*, by oxidising a pair of closely spaced thiols on the catalytic subunit to a disulfide, reversibly, in brain tissue.

This volume asks whether that surviving arm can be promoted. A broad afferent with one demonstrated direct arm is not a trigger. A single upstream event that reaches the same enzyme by three chemically unrelated routes would be. So the question is put in its strongest form and then tested: is there one event — amyloid- β , acting through the speck and the burst it licenses — that reaches the phosphatase by covalent oxidation, by demethylation and by induction of an inhibitor, all at once? And if the answer is yes, why has nobody measured it in a human Alzheimer neuron?

The answer is permitted to be no. It is worth stating the standard before the evidence, because the incentive in a synthesis runs the other way. *The Four Locks* removed a lock, removed a protease from a role it had never held, and demoted a cell type from a unit to a shorthand; subtraction is a legitimate result and is to be preferred to a joint softened until it passes. The outcome this volume actually reaches is a subtraction of exactly that kind, and it arrives not because the evidence is sparse — on one of the three routes it is unusually good — but because a crystal structure says the strongest route acts on the wrong subunit.

The discipline is the same as before. A connection must be shown and not inferred from adjacency. A protein raised in a disease and an enzyme inhibited in the same disease are not thereby inhibitor and enzyme. Two results in successive sentences of a review are not a pathway. And a result obtained in a neuroblastoma line is a result obtained in a neuroblastoma line: SH-SY5Y is not a neuron, N2a is not a neuron, HEK293 is not a brain, and a glioblastoma line is not even the right lineage. Where an arm rests on one of those, we say which one.

Sections II and III size the claim and fix the enzyme. Sections IV to VI take the upstream apart. Sections VII to XII take the three routes one at a time. Section XIII asks whether they converge, Section XIV grades every joint, Section XV states what would falsify each claim, and Section XVI names the experiment.

II. What Is Already Claimed

The first obligation of a synthesis is to establish that it is not restating a review. On the general proposition of this volume, it very nearly would be.

The general thesis has been reviewed, and reviewed well. *Regulation of the phosphoprotein phosphatase 2A system and its modulation during oxidative stress: a potential therapeutic target?* appeared in *Pharmacology & Therapeutics* in 2019 (Elgenaidi and Spiers, 2019). It presents an overview of the phosphatase system, then an analysis of its regulation by endogenous inhibitors, by post-translational modification and by microRNA, then a detailed critique of the data implicating reactive oxygen species, ischaemia, ischaemia–reperfusion and hypoxia in regulating the holoenzyme and its associated regulators, and finally the pharmacological options. It covers CIP2A and SET by name. It proposes, as therapy, either small-molecule activators of the phosphatase or CIP2A/SET antagonists. Its stated conclusion is that there is extensive evidence that oxidative stress modulates multiple components of this system, that "most of the data pertains to the catalytic subunit of PP2A," and that "irrespective of the underlying aetiology, free radical-mediated attenuation of PP2A activity is an emerging theme. However, in many instances, a dichotomy exists, which requires clarification and mechanistic insight."

Three things follow, and the volume is built on all three.

The first is prohibitive. **No part of this volume may claim that reactive species inhibit this phosphatase as an unclaimed idea.** It was claimed, surveyed, and qualified seven years ago, and the qualification — the dichotomy — is the honest reading. Where this volume finds an effect running in the opposite direction from the one the list implies, it is confirming that review's caution rather than discovering something.

The second is an opening, and it is the review's own admission. Most of the data pertains to the *catalytic subunit*. That is precisely the level at which a claim about this enzyme is least informative, because the catalytic subunit alone has almost no substrate selectivity worth the name and the noun "PP2A" names a family of roughly ninety-six holoenzymes rather than a molecule. A literature that has established oxidative regulation of the catalytic subunit has not thereby established oxidative regulation of the tau phosphatase, and the distinction is the axis along which this volume's three routes separate. Section III sets it up and Section XIII spends it.

The third is that the review is not about a neuron and not about this disease. Its frame is cancer, heart disease and inflammatory conditions; its title question is therapeutic; and its ischaemia–reperfusion material is cardiac. The vertebrate brain appears in it, but Alzheimer disease is not its subject. So what remains available is the neuron, the disease, and the measurements nobody has made in either.

What this volume claims, stated flatly so that it can be checked. Four things, none of them the general thesis.

One. Every human measurement of phosphatase activity in Alzheimer brain was made under reducing conditions, so the redox component of the deficit was reversed before it was counted; and an independent reductive shift occurs in brain vicinal thiols during the global ischaemia of death itself. The canonical deficit is the non-redox deficit, and the total is unmeasured.

Two. No study has measured PME-1, CIP2A, LCMT-1, the B α subunit and the redox state of the catalytic subunit in the same human Alzheimer tissue, let alone in the same neurons. The arms of this mechanism have never been weighed against one another in one piece of brain.

Three. Two regulators of the same catalytic subunit leave the same compartment in this disease by unrelated mechanisms, and the observation has not been made on one page. Section X makes it, and finds that what the two departures share is a loss in the nucleus rather than a gain in the cytoplasm.

Four. One experiment, named and costed, would settle the first of these and could kill the redox arm outright.

The corrections inherited from the companion volumes, stated compactly. These are settled in this corpus. They are not reopened below, and nothing in this volume may contradict them without explicitly reopening and re-adjudicating them.

Phosphorylation of the catalytic subunit at tyrosine 307 is **withdrawn** as a physiological inactivating event. The site was assigned in 1992 by inference from a carboxy-terminal truncation mutant, never confirmed by sequencing or mass spectrometry, and the commercial antibodies on which more than one hundred and eighty studies rest bind the unphosphorylated subunit with equal affinity and are eighty to ninety per cent blocked by methylation at leucine 309 (Frohner and colleagues, 2020a; Mazhar and colleagues, 2020). An apparent rise in that signal in Alzheimer tissue should be read as a *fall in methylation*. This matters more here than it did there, because Section XII re-reads that literature as methylation data and thereby recovers something from it.

The corrected tyrosine sites are **tyrosine 127 and tyrosine 284**. Src phosphorylates both; Fyn phosphorylates tyrosine 284 only; phosphorylation there evicts the B α subunit and raises tau phosphorylation at serine 202 (Sontag and colleagues, 2022). Neither has been measured in Alzheimer tissue.

Immunoprecipitation-based activity assays built on carboxy-terminus-directed monoclonal antibodies — clones 1D6, 7A6, G-4, 52F8 and polyclonal 2038 — are unreliable: they preferentially recognise the non-methylated catalytic subunit, fail to co-precipitate trimeric holoenzymes containing B or B' subunits, and in some cases recognise protein phosphatase 4

(Frohner and colleagues, 2020b). Every activity measurement cited in this volume is flagged where it used that route.

Calpain does not cleave SET. Oxidative stress does not activate asparaginyl endopeptidase; cysteine 219 oxidation deranges its maturation. The aldehydes of lipid peroxidation inhibit calpain rather than activating it, and the route from reactive species to calpain runs through glutathionylation of the ryanodine receptor and calcium. The pyramidal neuron is rejected as the unit of vulnerability. The retracted 2012 paper joining amyloid- β oligomers, the prion protein–Fyn complex and pathological tau is not cited. And saracatinib's phase 2a trial was negative on its primary metabolic endpoint and on every secondary clinical endpoint.

III. The Enzyme and the Assay

Nothing below can be graded without two facts about the object being graded, and both were established in the companion volume. They are restated compactly here because this volume's answer turns on them entirely.

The noun has to be the holoenzyme. The enzyme is a heterotrimer: a horseshoe scaffolding subunit A, a catalytic subunit C that performs the chemistry with two active-site metal ions, and one variable regulatory subunit B drawn from four structurally unrelated families — B or B55 or PR55, B' or B56, B'' or PR72, and the striatins. Fifteen genes and at least twenty-three isoforms populate the B position, and combinatorial assembly produces on the order of ninety-six distinct holoenzymes with non-redundant functions (Sontag and Sontag, 2014). The catalytic subunit alone has essentially no substrate selectivity; the B subunit is the enzyme's address book. The investigators who have worked hardest on its regulation state that one should "refrain from using ambiguous and imprecise statements, such as 'PP2A regulates this'."

One B subunit makes the enzyme a tau phosphatase, and it is B α . The holoenzyme containing the B55 α subunit, written AB α C, binds tau directly at a domain indistinguishable from its microtubule-binding domain (Sontag and colleagues, 1999); the crystal structure of the heterotrimer shows a seven-bladed β -propeller with an acidic substrate groove, and a fully reconstituted assay showed that B α "specifically and markedly facilitates dephosphorylation of phospho-tau" (Xu and colleagues, 2008). Displacing B subunits from the core enzyme is functionally equivalent to inhibiting it while leaving catalysis intact (Sontag and colleagues, 1996). And the assembly requirement is subunit-family-specific: methylation of leucine 309 is required for assembly of B55-family holoenzymes, while B' and B'' assembly carries no absolute methylation requirement (Longin and colleagues, 2007).

Those two facts together are the instrument this volume uses. They convert the question "does this route inhibit PP2A?" into a question that can actually be answered — *which*

holoenzyme does this route act on, and is it the one that erases tau? Applied to the three routes below, the instrument separates them cleanly, and the separation is the volume's principal finding. It also explains why the separation has not been made before: the assay in general use cannot see it.

The assay problem, and why a redox lesion is the hardest of all for it. Most published measurements of phosphatase activity immunoprecipitate the catalytic subunit with a carboxy-terminus-directed monoclonal and assay what comes down against a generic phosphopeptide. Those reagents preferentially recognise the non-methylated catalytic subunit, do not co-precipitate trimeric holoenzymes containing B or B' subunits, and in some cases recognise protein phosphatase 4 (Frohner and colleagues, 2020b). An assay built that way samples a demethylated, B-subunit-poor, partly heterologous subpool and reports its activity as the cell's. If the disease lesion is loss of a regulatory subunit, the assay discards the lesion during the immunoprecipitation.

A redox lesion is worse off still, and for an additional and independent reason. An assembly lesion at least survives into the tube and is then thrown away; a disulfide does not survive into the tube at all, because the buffer reduces it. So the two commonest lesion types in this volume are invisible to the standard assay by two different mechanisms — one is discarded by the antibody, the other is chemically undone by the buffer — and the residual number the field quotes is what is left after both. Section IX develops the second of these into the volume's methodological claim. It is entered here so that every activity figure quoted below is read with the right error bar, which is not a statistical one.

A corollary that constrains the whole volume. Because the standard assay is blind to assembly and blind to redox, the literature's convergence on a modest phosphatase deficit in Alzheimer brain may reflect the assay's floor rather than the disease's magnitude. The measurements that have proved durable are the ones that avoided this route: direct assay of tau dephosphorylation in brain extract, and immunohistochemical and immunoblot quantification of the holoenzyme itself. Where a claim below rests on an immunoprecipitation-based activity number, it is graded as resting on one.

IV. Amyloid- β as Three Different Reagents

The list names amyloid- β once, and the single name does most of the damage in the argument that follows from it. In the three routes of this volume, amyloid- β appears in three chemically unrelated capacities, and a proposition that is well supported for one of them is not thereby supported for the others. This section separates them before anything is built on top.

As a ligand, it assembles an inflammasome. In this capacity amyloid- β is not a chemical reagent at all; it is a particulate stimulus recognised by a microglial sensor, and everything

downstream — the speck, the caspase, the interleukin — is the cell's own machinery. The relevant evidence is immunological and the relevant cell is not a neuron. Section V takes it up.

As an oxidant, it makes chemistry happen. Amyloid- β coordinates redox-active transition metals and, in the presence of a reductant, participates in the production of hydrogen peroxide; its aggregates initiate membrane lipid peroxidation and generate the α,β -unsaturated aldehydes 4-hydroxynonenal and acrolein, which then form Michael adducts on protein histidine, lysine and cysteine residues. In this capacity the peptide is a source of oxidising equivalents, and the cell in which it acts is whichever cell its membranes are nearest. This is the capacity the disulfide arm of Section VIII actually requires.

As a kinase driver, it acts through receptors. Soluble assemblies bound to cellular prion protein activate Fyn through metabotropic glutamate receptor 5, driving NR2B phosphorylation and spine loss; this is a signalling event with a receptor, a coupler and a kinase, and it has nothing to do with the peptide's metal chemistry.

And there is a fourth capacity the list does not name, which turns out to matter most. In the CIP2A arm of Section XII, amyloid- β acts as an agent of DNA damage: the demonstrated chain in Alzheimer tissue runs from amyloid- β to DNA damage to checkpoint kinase 1 to CIP2A, and not from amyloid- β to reactive species to CIP2A (Hu and colleagues, 2022; Wang and colleagues, 2024). That the two would be joined by *oxidative* base damage is the obvious supposition, and it is the one this volume declines to make on the field's behalf — though Section XII records that the reactive-species half of the bridge has been drawn once, in a primary neuron, and that the commissioning brief for this volume was wrong to call it absent.

Why the distinction is load-bearing rather than pedantic. The proposition under test is that one upstream event reaches the phosphatase by three routes. If amyloid- β reaches route one as an oxidant, route two as an oxidant, and route three as a mutagen, then the "one upstream event" is one *molecule* participating in three unrelated chemistries, which is a considerably weaker claim than the list implies — it is the claim that a peptide abundant in a diseased brain does several things, which nobody disputes. A shared trigger requires a shared *mechanism* of triggering, not a shared reagent, and Section XIII holds the argument to that standard.

One further discipline, inherited. *The Four Locks* applied reagent scepticism to the phospho-tyrosine-307 antibody and found twenty-eight years of literature resting on a reagent that reported something else. The same scepticism is owed to any claim of neuronal inflammasome components below: where the claim is that a neuron expresses NLRP3, the clone is named or the claim is not made.

V. The Speck

The ASC speck is the most concrete object in this volume, and the part of the story with the best-established biology. It is also the part furthest from the neuron.

What it is. On activation of the NLRP3 sensor, the adaptor protein ASC — apoptosis-associated speck-like protein containing a caspase recruitment domain, the product of *PYCARD* — polymerises into a single large perinuclear aggregate, typically one per cell, which serves as the platform on which pro-caspase-1 is recruited and activated, cleaving pro-interleukin-1 β to its mature form. The speck is a prion-like polymer: it nucleates, it grows, and it is stable. When the cell dies by pyroptosis the speck is released intact into the extracellular space, where it remains catalytically competent and can be taken up by neighbouring cells, propagating inflammasome activation from cell to cell.

What it does in this disease, and this is properly established. Microglia-derived ASC specks bind amyloid- β , and they cross-seed it. In Alzheimer brain and in mouse models, ASC specks are found in the extracellular space and within amyloid deposits; specks bind amyloid- β and increase its aggregation; intrahippocampal injection of ASC-speck-containing brain homogenates from an amyloid model induces amyloid pathology in a host animal, and this is blocked by an anti-ASC antibody and does not occur in ASC-deficient animals (Venegas and colleagues, 2017). This is a *Nature* paper with genetic and antibody-based controls and it is not in doubt.

And it reaches into the tau arm, though by a microglial route. Aggregated tau activates the NLRP3–ASC inflammasome, and inflammasome activation exacerbates both exogenously seeded and non-exogenously seeded tau pathology in vivo; the activation follows microglial uptake of tau seeds and their sorting to the lysosome (Stancu and colleagues, 2019). And the reverse arrow is a *Nature* paper of its own: loss of NLRP3 inflammasome function reduces tau hyperphosphorylation and aggregation "by regulating tau kinases and phosphatases," with intracerebral injection of fibrillar-amyloid- β -containing homogenate inducing tau pathology in an NLRP3-dependent manner (Ising and colleagues, 2019).

That last result is the keystone of the companion volume *The Silenced Eraser*, which reports from its figures that the inflammasome-bearing animals carried elevated levels of the methylesterase PME-1 and a corresponding shift of the phosphatase toward inactivity. *We have verified that study's abstract-level claim — that inflammasome loss acts by regulating tau kinases and phosphatases — and have not independently verified the PME-1 measurement against the published figures; readers relying on the PME-1 specificity should check it.* The distinction matters in Section X, because the inflammasome's reported effect on PME-1 is on its **abundance**, while the redox literature's effect is on its **compartment**, and those are two different mechanisms that must not be merged.

What the speck literature does not contain. Every demonstration above is a demonstration about microglia, or about what microglia do to the extracellular space, or about what happens to a mouse when microglia lack a sensor. In none of them is the measured object inside a neuron. The speck is made in a microglion, released from a microglion, taken up by a microglion or an astrocyte, and it acts on amyloid- β and on tau in the space between cells. The step the list requires — the speck reaching *into* the neuron, and specifically raising oxidising equivalents there — is a separate claim with a separate and far smaller evidence base, and Section VI assembles all of it.

A note on the cell-type question, which is worth making in detail because it is a reagent problem of the kind this corpus exists to catch. The *NLRP3* and *PYCARD* transcripts in human brain are overwhelmingly myeloid: in single-cell expression data, *NLRP3* runs at roughly sixty-six normalised counts per million in microglia against under two in excitatory or inhibitory neurons, and *PYCARD* at roughly forty against one or below — ratios of about thirty-five to one and twenty-nine to one, with *CASP1* nearer three hundred to one. Both genes are classed as central-nervous-system-macrophage enriched. We record the honest complication that the residual neuronal signal sits somewhat above the ambient floor set by unambiguously microglial genes, and the countervailing one that single-nucleus sequencing is a poor instrument for exactly this class of gene.

At protein level the largest human study is decisive in the other direction from the neuronal claim: across twenty-three symptomatic Alzheimer, twenty-five preclinical and twenty-one control cases, **only microglia expressed all of the NLRP3 inflammasome-forming proteins**, with neurons showing a non-canonical caspase instead (Moonen and colleagues, 2023). A second human series reports NLRP3 mainly in microglia and states that it was not observed in the neuronal population.

And the neuronal claim is demonstrably reagent-dependent. The two flagship papers reporting neuronal NLRP3 — in Parkinson's disease and in an amyloid model — rest on the same single mouse monoclonal, **Adipogen clone Cryo-2 (AG-20B-0014)**, and we could not establish that either validated it on knockout tissue. Eight anti-NLRP3 antibodies used in a published disease literature were formally tested against three validation criteria and **none met all three in both mouse and human samples**; one produced bands also present in knockout tissue, and one gave a signal that was *up-regulated* rather than absent in knockout tissue (Kosmidou and colleagues, 2018). We could locate no published test of any anti-NLRP3 antibody on knockout brain tissue by immunohistochemistry. And in human Alzheimer brain the epitope has been shown to determine the answer: one laboratory reports that an antibody directed at the pyrin domain of ASC labelled neurons while a commercial antibody against the caspase-recruitment domain of the same protein primarily labelled microglia — a result whose authors have an interest in the first reagent, which they state.

Following the discipline of Section IV, this volume therefore does not make or rely on a claim of neuronal NLRP3 expression. Note that this is a constraint on the *mechanism* and not on the *phenomenon*: a speck does not have to be made in a neuron to act on one, and the arm of Section VI depends on the speck arriving from outside.

VI. Does the Speck Reach the Neuron?

This is the first joint of the spine, and it is the weakest. The claim required is that an ASC speck, made by glia, raises reactive oxidising equivalents inside a neuron. We assemble every direct demonstration we could locate, and there is one.

The one result. In a study of ethanol consumption in Alzheimer models, ASC specks produced by primary glia were applied to cells, and the authors report that "glia-produced ASC specks induce IL-1 β in microglia and astrocytes and induce ROS in SH-SY5Y neurons, contributing to sustained neuroinflammation" (Brezani and colleagues, 2025). That sentence is the entire direct evidential basis for this joint in the literature as we find it, and four things about it must be stated rather than smoothed.

It is one result in one cell line, and SH-SY5Y is not a neuron. It is a human neuroblastoma line, mitotic unless differentiated, karyotypically abnormal, and routinely used because it is convenient. *The Four Locks* rejected the pyramidal neuron as the unit of vulnerability on the grounds that morphological class does not predict which cells tangle; a neuroblastoma line is a weaker object than a morphological class.

The specks were produced by glia and applied. This is the right experiment for the claim — it does not require neuronal inflammasome expression, which is exactly the claim Section V declined to make — but it establishes that specks *applied* to a cell line raise reactive species in it, not that specks generated in a diseased brain reach a neuron in that brain.

The provenance is an alcohol study. The paper's subject is that repeated ethanol binges aggravate amyloid pathology and plaque burden in the hippocampus of APP/PS1 mice, that ethanol adds to NLRP3 activation, interleukin-1 β release and ASC aggregation in vivo and in primary glia, and that ethanol potentiates the effect of amyloid- β on inflammasome activation in primary astrocytes and microglia. The neuronal reactive-species result is the last item in that sequence. It is not a smaller result for appearing there, but a reader of the corpus is entitled to know that the only arrow from the speck to a neuron-like cell was drawn in a study about binge drinking, and that the field has not gone back to it.

And the mechanism of entry is unestablished. We searched specifically, and at length, for a demonstration that an extracellular ASC speck is internalised by a neuron, and found none in any

system. What is established is uptake by **macrophages**, where phagocytosis of specks induces lysosomal damage and nucleation of soluble ASC in the recipient cell (Franklin and colleagues, 2014; Baroja-Mazo and colleagues, 2014); by **microglia**, where released ASC is functionally built into the neighbouring cell's inflammasome (Friker and colleagues, 2020); and by two non-neural cell types, coronary artery smooth muscle and hepatic stellate cells. *Astrocytic* internalisation, which we had expected to find, we could not verify either. The most recent dedicated review of neuronal inflammasomes, written by the group that has done most of the neuronal ASC work, makes no claim that neurons internalise specks.

The consequence for the one positive result is that its mechanism is open in three directions: the reactive species may arise from an internalised speck, from engagement at the neuronal surface, or from something in the conditioned medium accompanying the speck preparation. The published neuronal arm is a set of plate-based viability and reactive-species assays; there is no labelled speck and no imaging of entry.

One mis-citation to avoid, because it is easy to make. A human study reporting neuronal ASC immunoreactivity with a pyrin-domain-directed antibody cannot distinguish neuronally expressed ASC from internalised ASC, and its authors make no uptake claim. It should not be cited as evidence of neuronal speck uptake.

The counter-observation, and it must not be buried. A study of cerebellar resistance in the 5xFAD model reports that the cerebellum has minimal amyloid- β deposition despite robust transgene expression, lacks the dystrophic synaptic accumulations prominent in cortex, and — "paradoxically" in the authors' own word — contains **more** abundant ASC inflammasome specks than the cortex (Cangalaya and colleagues, 2026). Extracellular matrix proteoglycans brevican and aggrecan were elevated in the 5xFAD cerebellum, and cerebellar microglia showed enhanced internalisation of brevican alongside small amyloid- β aggregates, exceeding that of cortical microglia.

Two things follow, and the first is a correction to how this volume was commissioned. The resolution — that rapid clearance prevents these seeds from driving plaque formation — is **the authors' own stated interpretation**, offered in their abstract, not an adjudication performed here. This volume reports it rather than claiming it.

The second is the consequence for the joint, and it cuts against the spine. If speck abundance can be higher in a region that develops less pathology, then speck abundance is not sufficient for downstream damage, and an argument that runs from more specks to more neuronal oxidative stress to more phosphatase inhibition has lost its monotonicity. That is a real weakening and it is entered as one.

What it does not do is disturb the cerebellar exemption as *The Four Locks* used it.

That volume used the cerebellum as an internal negative control for the *phosphatase* deficit — the AβC holoenzyme is reduced in Alzheimer cortex and not in Alzheimer cerebellum, and the endogenous inhibitor's redistribution likewise spares cerebellar granule and Purkinje cells (Sontag and colleagues, 2004a; Tanimukai and colleagues, 2005). Those are measurements of a different molecule, in a different species, in human autopsy tissue rather than a transgenic mouse. A mouse cerebellum rich in specks and poor in plaques does not speak to whether a human cerebellum is spared a phosphatase deficit. The two observations are compatible and neither settles the other.

We record, however, that the exemption was already carrying internal tension before this observation arrived, and a sequel should say so. In the methylenetetrahydrofolate-reductase-deficient mouse the loss of methyltransferase and methylated catalytic subunit is *primarily in hippocampus and cerebellum*; and in human tissue 4-hydroxynonenal is significantly increased in the cerebellum at the stage of mild cognitive impairment. The cerebellum is exempt from tangles and from the holoenzyme deficit. It is not exempt from oxidative stress, and it is not exempt from perturbation of the methylation machinery. For a volume about oxidative stress and methylation, that is the relevant fact, and it means the cerebellum is a weaker internal control here than it was there.

Grade: Weak. One result, one neuroblastoma line, one study whose subject is ethanol, no demonstrated mechanism of neuronal entry, and a regional observation in which speck abundance and pathology run in opposite directions. The honest statement is that the speck is an established upstream of amyloid seeding and of microglial and astrocytic inflammasome propagation, and a hypothetical upstream of neuronal oxidative stress. Section XIII does not permit this joint to carry weight, and Section XV names what would strengthen it.

VII. The Oxidative Environment of the Alzheimer Neuron

Before asking whether oxidation reaches a particular enzyme, it is worth establishing what is actually oxidised in human Alzheimer tissue, because the answer is specific and it does not include the enzyme.

Oxidative damage is present, and its timing is the most useful thing about it.

Neuronal oxidative damage was established in situ with antibodies against oxidised guanosine, showing prominent cytoplasmic immunoreactivity in neurons of hippocampus, subiculum, entorhinal cortex and neocortex, significantly increased in Alzheimer cases, and greatly diminished by ribonuclease but only slightly by deoxyribonuclease — so the damage is predominantly to RNA, and its cytoplasmic predominance implicates mitochondria as the source (Nunomura and colleagues, 1999). The timing result is the one that licenses this whole line of argument: oxidative

damage is quantitatively greatest **early** and decreases with disease progression; increased amyloid- β deposition is associated with *decreased* oxidative damage; and neurons containing neurofibrillary tangles show a forty to fifty-six per cent *decrease* in relative oxidised guanosine compared with tangle-free neurons (Nunomura and colleagues, 2001). Whatever else is true, oxidative damage is upstream of the tau lesion in time, which is the licence for treating it as a candidate trigger rather than a consequence.

Lipid peroxidation shows the same early profile. Free-radical-derived isoprostanes are significantly increased in frontal, parietal and occipital lobes in mild cognitive impairment and in late Alzheimer disease, with no significant difference between those two stages (Markesbery and Lovell, 2005); 4-hydroxynonenal is significantly increased in hippocampus and parahippocampal gyrus, superior and middle temporal gyrus and cerebellum in mild cognitive impairment, and acrolein in temporal cortex, again with no significant difference between mild impairment and early disease (Williams, Lynn and Markesbery, 2006). The lesion is present before dementia and does not worsen with it. As a control on over-reach, peripheral isoprostanes are *not* increased in this disease (Montine and colleagues, 2002); the signal is restricted to brain and cerebrospinal fluid.

Oxidative damage to DNA is separately established, and it is the arm Section XII needs. The Nunomura measurements above are of RNA, and the distinction matters because the CIP2A route runs through DNA. The DNA evidence is independent and is chemically rigorous: a threefold increase in 8-hydroxy-2'-deoxyguanosine in mitochondrial DNA of Alzheimer parietal cortex with a smaller increase in nuclear DNA (Mecocci, MacGarvey and Beal, 1994); by gas chromatography-mass spectrometry with stable-isotope internal standards, significant elevations of 5-hydroxycytosine, 5-hydroxyuracil, 8-hydroxyadenine and 8-hydroxyguanine in Alzheimer brain, with **no significant correlation between the oxidised bases and tangle or plaque counts** (Gabbita, Lovell and Markesbery, 1998); and, decisively for timing, the same chemistry showing that oxidative damage to nuclear and mitochondrial DNA occurs in amnesic mild cognitive impairment, "the earliest detectable phase" of the disease (Wang, Markesbery and Lovell, 2006). An independent laboratory found increased levels of **some but not all** oxidised bases across seven regions, which is the appropriate caution. Double-strand breaks are also raised: focal γ H2AX labelling is increased in neurons and astrocytes in hippocampus and frontal cortex from mild cognitive impairment onward, with the authors establishing that pan-nuclear labelling does *not* indicate breaks (Shanbhag and colleagues, 2019).

So the substrate for the CIP2A arm exists in human tissue, early, and in the right direction. What does not exist is the link from it to the kinase, and Section XII holds that line.

What is oxidised, and it is not this enzyme. The systematic redox proteomics of mild cognitive impairment and early Alzheimer brain identified the proteins carrying 4-hydroxynonenal adducts, and the list is overwhelmingly of glycolytic and mitochondrial energy enzymes — enolase,

lactate dehydrogenase, phosphoglycerate kinase, adenosine triphosphate synthase, pyruvate kinase, triosephosphate isomerase, malate dehydrogenase, manganese superoxide dismutase — with measured activity losses in several, and neither tau nor any phosphatase subunit among them (Reed and colleagues, 2008; Reed and colleagues, 2009). *The Four Locks* recorded this correctly as a statement about that survey's detection rather than about the enzyme's chemistry, and this volume keeps that qualification. But it also draws the consequence, which that volume left implicit: **the detected burden of lipid peroxidation in human Alzheimer tissue routes to energy failure and not to the components of this mechanism.** A volume proposing that lipid peroxidation disables a phosphatase has to explain why the only unbiased human survey of lipid-peroxidation targets did not find it.

Three answers are available and they are not equally good. The survey looked for *irreversible* Michael adducts, and the lesion proposed here is a *reversible* disulfide, which no adduct-detection method would find — this is the best answer and it is the reason the experiment of Section XVI is a redox-shift assay and not a proteomics re-run. The enzyme may be below the survey's detection floor, which is possible and unfalsifiable as stated. Or the enzyme is genuinely not a target of lipid peroxidation in human tissue, which is the null this volume must be prepared to accept.

One substrate-side effect belongs here rather than in the enzyme's column. Exposure of cultured hippocampal neurons to 4-hydroxynonenal made tau *resistant to dephosphorylation*: the aldehyde produced a moderate rise in basal tau phosphorylation and prevented dephosphorylation by alkaline phosphatase in neurons pretreated with okadaic acid, with antibody evidence of the aldehyde binding directly to tau (Mattson and colleagues, 1997). This is a modification of the substrate and not a demonstrated inhibition of the phosphatase, and it must be cited as such — but it produces the same phenotype from the other end. A volume that finds the enzyme unmodified and the substrate modified has found something, and it is not what the list predicted.

The chemistry has to be exact from here on. The remainder of this volume distinguishes four things that the word "oxidation" is routinely made to cover, and the distinction determines what is reversible and therefore what a cell can undo and a buffer can undo. A **disulfide** between two cysteine thiols is a two-electron oxidation, reversible by thiol–disulfide exchange with any reductant. A **sulfenic acid** is a two-electron oxidation of a single thiol, reversible, and unstable. A **sulfinic acid** is reversible only by sulfiredoxin and only on peroxiredoxins; a **sulfonic acid** is not reversible at all. **S-glutathionylation** is a mixed disulfide with glutathione, reversible by glutaredoxin. And **Michael adduction** by 4-hydroxynonenal or acrolein is a covalent addition, effectively irreversible on the protein's lifetime.

These are not interchangeable species of "oxidative stress," and the routes of this volume distribute across them unevenly: the arm of Section VIII is a reversible disulfide, the human survey

above detected irreversible adducts, and S-glutathionylation — which is frequently asserted for this enzyme — is, as Section VIII shows, not demonstrated.

VIII. The Disulfide Bridge

This is the arm that survived *The Four Locks*, and it is the arm this volume was written to promote. It does not survive promotion intact. The evidence base is larger than the companion volume knew — nine papers rather than three — and the two the companion volume did not carry both cut against it.

The programme, and whose it is. Essentially the whole of this literature is the work of one laboratory, Timothy Foley's at the University of Scranton, across two decades. That is not a criticism; it is a fact about replication that a reader is entitled to have stated, and it means the arm has never been independently reproduced by a second group in brain tissue.

The positive results, in order. In rat brain subcellular fractions, the constitutively expressed phosphatase activity directed at a dual-phosphorylated extracellular-regulated-kinase activation-domain peptide was attributable "mainly, if not entirely" to protein phosphatase 2A, and hydrogen peroxide — *and glutathione disulfide* — inhibited that activity by a dithiothreitol-reversible mechanism (Foley, Armstrong and Kupchak, 2004). This is the physiological-oxidant version of the result and it is the strongest single entry in the arm, because glutathione disulfide is a species the cell actually generates rather than a laboratory arsenical.

Treatment of the rat brain soluble fraction with a thiol-reactive biotinylating agent produced substantial phosphatase inhibition and caused the catalytic subunit to bind an immobilised avidin column; both effects were fully reversed by dithiothreitol, and phosphatase specific activity in the reduced eluate was up to sevenfold higher than in the starting soluble fraction (Foley and Kintner, 2005). In rat cerebral cortex high-speed supernatant, phosphatase activity showed substantial reducing-agent-reversible inhibition, associated both with intermolecular disulfide cross-linking of the catalytic subunit to other brain proteins and with an apparent novel intramolecular disulfide within the catalytic subunit; the vicinal dithiol cross-linker phenylarsine oxide produced potent, dithiothreitol-reversible inhibition (Foley and colleagues, 2007). And a refined phenylarsine-oxide affinity method showed the catalytic subunit forming stable, reducible adducts with or without associated regulatory subunits, with a fraction of catalytic subunit still carrying disulfides after stringent anti-artefact homogenisation, suggesting that a population of the enzyme carries disulfides in vivo (Foley and colleagues, 2011).

A methodological result from the same programme that the experiment of Section XVI depends on. Capture of readily oxidisable vicinal-thiol proteins by immobilised phenylarsine oxide requires a reducing agent that restores the thiols without itself competing for the

arsenical — which conventional thiol reductants do. Substituting tris(2-carboxyethyl)phosphine, which contains no thiol group, greatly improved capture from rat brain extract (Foley and colleagues, 2010). Anyone repeating this chemistry in human tissue must use a phosphine and not dithiothreitol, and this is the paper that establishes why.

The first result that cuts against it, and it is from the same laboratory. A survey of protein vicinal thiol oxidation in healthy rat brain, with brains frozen rapidly after euthanasia to preserve in vivo redox states and alkylation applied on tissue disruption to trap reduced thiols, defined brains as Reduced or Moderately Oxidized by their glutathione couple and asked which proteins carried vicinal-thiol disulfides. Levels of such disulfides — *but not protein S-glutathionylation* — were higher in the Moderately Oxidized brains (Foley and colleagues, 2014). The follow-up identified the proteins: the catalytic vicinal thiols of peroxiredoxins 1 and 2 as the peroxide sensors, and, among non-peroxidase proteins showing prominent vicinal thiol oxidation, the catalytic subunit of sodium/potassium adenosine triphosphatase, tubulins, glyceraldehyde-3-phosphate dehydrogenase, and **protein phosphatase 1** (Foley, Katchur and Gillespie, 2016). Protein phosphatase 2A is not on that list.

Two consequences follow and both belong in the ledger rather than a footnote. **S-glutathionylation of the catalytic subunit is not demonstrated**, and was specifically found low and unchanged in the tissue where the disulfides were found — so the frequently encountered claim that this enzyme is regulated by glutathionylation should not be made. And **the same laboratory's in vivo survey of exactly this chemistry, in exactly this tissue, did not recover this enzyme**. The most parsimonious reading is that the phosphatase's vicinal thiols are demonstrably oxidisable when an oxidant or an arsenical is applied to an extract, and are not among the proteins whose vicinal thiols are preferentially oxidised in a living brain under physiological redox variation. That is a real distinction and it is the difference between a mechanism and a lesion.

The second result that cuts against it is newer still, and it cuts twice. Using the same phenylarsine-oxide affinity chemistry under conditions designed to trap in vivo thiol states, the laboratory reported that creatine kinase B and α -enolase carried preferentially oxidised vicinal thiols relative to other proteins including neuron-specific enolase; that vicinal-thiol-linked redox states were **stable over the lifespan of rats**; and that decapitation-induced global ischaemia produced a **transient reductive shift** in these couples (Foley and colleagues, 2024).

The first of those is a problem for the disease argument. A redox couple that does not change across the rat lifespan is a poor candidate for the mechanism of an age-associated disease, and the burden moves to showing that the disease does what ageing does not. The second is a problem for the measurement, and it is the more interesting of the two: Section IX develops it.

The residue problem, stated precisely. In none of the brain-tissue studies were the cysteines identified by number. They are defined *operationally* — as vicinal, and as phenylarsine-oxide-reactive — which is a functional definition and not a structural one. Residue-level assignment for this enzyme exists, but only for a different chemistry: two thiol-reactive biotinylated electrophiles were applied to purified AB α C and AB δ C holoenzymes, both alkylated all three subunits, **only one of the two inhibited catalytic activity**, and the inhibitory one alkylated **four cysteine residues in the catalytic subunit** that the non-inhibitory one did not touch, with a homology model built on protein phosphatase 1 relating specific adducts to inhibition (Codreanu and colleagues, 2006). *The abstract does not number those four residues and we have not verified them against the full text; anyone building on the residue assignment should read the paper's figures rather than this sentence.*

So the position is: the enzyme is an established target of cysteine-directed electrophiles at named-but-here-unverified residues, in a human embryonic kidney expression system, by an irreversible chemistry; and it is an established target of reversible disulfide formation at unnamed residues, in rat brain extract. **The two bodies of evidence do not overlap, and neither is the one the disease argument needs.** Section XVI's experiment is designed to produce the missing one.

The candidate pair can nevertheless be named, and naming it is worth doing because it makes the arm falsifiable. The human catalytic subunit carries exactly ten cysteines — at residues 20, 32, 50, 55, 133, 165, 196, 251, 266 and 269 — and cysteines 266 and 269 sit in a CXXC motif. In the solved structures of the core enzyme and of the holoenzyme, the sulfur atoms of cysteines 266 and 269 lie approximately four and a half to five and a half ångström apart, and **no other cysteine pair in the protein comes within nine ångström of another.** On the structures, therefore, the phosphatase has exactly one vicinal thiol pair, and it is this one. Cysteine 269 is independently the residue to which the covalent inhibitors fostriecin and phoslactomycin bind and which microcystin requires, and it sits in the β 12– β 13 loop nearest the catalytic metals. A modification there would be expected to interfere with catalysis, which is what the arm requires.

Three honesty conditions attach to that paragraph and none may be dropped. The identification of cysteines 266 and 269 as *the* redox switch is a **structural inference made in a review**, which cites the brain-tissue papers that name no residue; it is not a measurement, and it should be cited as an inference. A separation of four and a half to five and a half ångström is not a disulfide — a disulfide is about two ångström — so forming one requires a local rearrangement that no structure shows. And a claim that circulates in this literature, that cysteines 50 and 55 constitute a second vicinal pair because they are five residues apart in sequence, is **structurally false**: their sulfurs are more than fifteen ångström apart in the solved holoenzyme, and the claim should be withdrawn wherever it appears.

The value of naming the pair is that it converts an operational definition into a testable one. A mass-spectrometric experiment on the catalytic subunit from oxidised brain extract, looking specifically for a 266–269 disulfide-linked peptide, would either assign the reversible modification or show that the operationally defined vicinal thiols are not the structurally obvious ones. Nobody has done it.

And the species problem, which is total. There is no human tissue anywhere in this arm. There is no Alzheimer tissue anywhere in this arm. Every result above is rat brain, rat brain extract, or purified enzyme. The corroborating work outside brain — hydrogen peroxide reducing okadaic-acid-sensitive phosphatase activity by eighty to eighty-five per cent in epithelial monolayers with phosphatases 1 and 2C unaffected, and oxidised glutathione inhibiting the enzyme reversibly (Rao and Clayton, 2002) — is epithelial. The nearest approach to a neuron is hydrogen peroxide inhibiting the enzyme in pheochromocytoma, neuroblastoma and primary murine neurons, abrogated by N-acetylcysteine (Chen and colleagues, 2009), which is an activity measurement and not a thiol measurement, and therefore does not establish the mechanism.

Grade: Moderate, downgraded. *The Four Locks* graded this arm **Strong (biochemical, brain tissue)** on the three papers it carried, with a caveat about residue assignment. On the full nine-paper record the grade cannot be sustained at that tier. The demonstration that the enzyme is reversibly inhibited by vicinal thiol oxidation in brain extract is solid and replicated within one laboratory. The demonstration that this happens in a living brain is absent; the survey that looked for it in a living brain recovered four other proteins and this one's close relative, protein phosphatase 1, instead; the redox couple in question does not shift with age; glutathionylation is excluded rather than supported; the residues are unassigned; and no human or diseased tissue has been examined. **Moderate** is the honest tier, and the downgrade is entered as a correction to the companion volume in Section XIV.

IX. The Buffer

The Four Locks closed its oxidative-stress section with a claim it did not verify, and this volume was commissioned partly to verify it. The verification did not succeed, and the reason it did not succeed is itself the finding. The claim has to be restated, and restated more weakly — and then, on independent evidence, it acquires a second arm that is stronger than the first.

The claim as inherited. That volume wrote: "Every measurement of phosphatase activity in Alzheimer brain assembled in Section III was made in homogenisation buffer containing reducing agent — β -mercaptoethanol at ten millimolar in the standard buffer of the laboratory that produced most of them." The inference drawn from it was that any disulfide-based component of the deficit

was reversed in the tube before the assay began, so that the canonical figure of approximately thirty per cent is a measurement of the *non-redox* deficit.

What the primary methods actually yield. We attempted to recover the homogenisation and assay buffer, paper by paper, from the published methods of every primary study reporting a phosphatase activity measurement in human Alzheimer brain. The result is the table below. In four of the six papers that carry the canonical observations the buffer is **not recoverable** from anything we can read: none has a PubMed Central deposit, and the two nominally free publisher files could not be retrieved through any of six routes attempted.

Table 1 — Homogenisation and assay conditions in the primary human phosphatase-activity literature.

Study	Tissue	Buffer	Reductant	Reported result	Source
Gong and colleagues, 1993	Frontal grey and white matter; 13 AD, 12 controls	Unrecoverable	Unrecoverable	Type 2A activity significantly lower in grey and white matter; no percentage given	Abstract only
Gong and colleagues, 1995	AD brain; region not stated in abstract	Unrecoverable	Unrecoverable	Tau phosphatase activity "decreased by approximately 30%"; substrate AD-derived hyperphosphorylated tau, Tau-1 sites	Abstract only
Liu and colleagues, 2005	Human brain, AD and control	Unrecoverable	Unrecoverable	PP2A $\approx$ 71% of total tau phosphatase activity; PP2A and PP5 decreased, PP2B increased	Abstract only
Sontag and colleagues, 2004a	Frontal and temporal cortex	Unrecoverable	Unrecoverable	AB α C reduction "matched the decrease in PP2A activity measured in the same brain homogenates"; no number	Abstract only
Sontag and colleagues, 2004b	AD-affected regions	Unrecoverable	Unrecoverable	No activity assay reported at abstract level	Abstract only
Pei and colleagues, 1998	Superior temporal cortical grey matter, subcellular fractions	Unrecoverable	Unrecoverable	No significant difference in any phosphatase activity between AD and control ; PP2A and PP2B <i>protein</i> increased in postsynaptosomal S2	Abstract only
Basurto-Islas and colleagues, 2013	Human frontal cortex for blots; activity assayed in rat brain slices	50 mM Tris-HCl pH 7.4, 8.5% sucrose, 2 mM EDTA, 2 mM EGTA, 10 mM β-mercaptoethanol , 1, benzamidine, AEBSF, pepstatin A, aprotinin, leupeptin	β -mercaptoethanol, 10 mM homogenisation; 20 mM in the assay	Not a human activity measurement	Full text

Study	Tissue	Buffer	Reductant	Reported result	Source
Arif and colleagues, 2014	Guam parkinsonism -dementia, frontal cortex	Same recipe, verbatim, at 9 mL buffer per g tissue	β -mercaptoethanol, 10 mM homogenisation; 20 mM in the assay	PP2A activity decreased; no numeric value in the supplement	Full text and supplement

What is therefore established, and what is not. It is established, verbatim and from two full texts, that the laboratory which produced most of the human observations uses a homogenisation buffer containing ten millimolar β -mercaptoethanol and a tau-phosphopeptide assay buffer containing twenty millimolar β -mercaptoethanol. It is **not** established that Gong 1993, Gong 1995, Liu 2005 or either Sontag 2004 used that buffer, and this volume does not import it into their rows. Gong 1993 in particular used an entirely different readout — ^{32}P -labelled phosphorylase kinase and a poly(glutamate, tyrosine) copolymer — so a shared recipe cannot be assumed even within the same laboratory.

The claim is therefore restated as a prediction, which is what it always was. If the four unrecoverable papers used the buffer their laboratory's other papers use, then the redox component of the deficit was reduced away before it was counted, and the canonical thirty per cent is the non-redox residue. That conditional is cheap to discharge: it requires a library, not an experiment. We state it as an open question rather than a finding, and we record that *The Four Locks'* unconditional version overstates what its own sources support. That correction is entered in Section XIV.

The second arm, which does not depend on any buffer. There is an erasure upstream of the tube, and it is the more interesting of the two because nobody chose it. In rat brain, measures of vicinal-thiol-linked redox state revealed "a transient reductive shift in these redox couples following decapitation-induced global ischemia" (Foley and colleagues, 2024). Every human brain in every study in Table 1 experienced global ischaemia before collection — that is what dying is — followed by an agonal period of unrecorded duration and a post-mortem interval of hours.

If the rat result holds in human tissue, the consequence is direct and it runs the same way as the buffer: **the disease's disulfides are partly reduced by the manner of death, and what survives that is reduced by the homogenisation buffer.** Two independent erasures of the same lesion, neither of them a decision anybody made, one of them not even a method. We are careful about what this does and does not say. It is a rat result, in healthy brain, after a clean decapitation rather than a human death from pneumonia; the direction is established and the magnitude in human tissue is entirely unknown; and a *transient* shift may have reversed before a post-mortem interval of hours has elapsed, which would remove the problem or invert it. It is a hypothesis with a mechanism, and Section XV states what would test it.

The negative result that nobody cites, and it is not in this corpus. Measuring the same phosphatases in the same disease in superior temporal cortical grey matter, with protein levels and activities determined in parallel across subcellular fractions, one study found that "because of the large variation from case to case, the activity levels of none of the phosphatases investigated were significantly different between the AD and control groups" — and that the *protein* levels of protein phosphatase 2A and of calcineurin were significantly **increased** in the postsynaptosomal fraction of the Alzheimer group, correlating with hyperphosphorylated tau (Pei and colleagues, 1998). Three of its seven authors are authors of the papers reporting the deficit.

This is a failed replication of the central human observation of this entire literature, published in a smaller journal, and it appears in neither companion volume. It must be entered, and it must be entered without being used. Its own authors interpret their null as compensation — that any attempt by the Alzheimer brain to compensate for decreased tau phosphatase activity remains unsuccessful — which reads the result through the hypothesis it fails to support. Its large case-to-case variance in a small series is a plain power problem. And a reader inclined to the argument of this section will notice that a real but redox-carried deficit, reduced away in the tube, would leave exactly a small non-significant residue swamped by inter-case variance. **That reading is available and this volume does not adopt it**, because a hypothesis that explains a null result as concealed evidence for itself is unfalsifiable in precisely the way Section XV exists to prevent. Pei 1998 is entered as what it is: a negative, from within the same tradition, that the field has not reconciled and that the corpus should have carried.

One complication that deserves its own paragraph, because it is uncomfortable.

The single paper whose full methods we could read, and from which the verified buffer recipe above is taken, is a paper whose central claim this corpus has withdrawn. Arif and colleagues report decreased phosphatase activity in Guam parkinsonism-dementia "associated with an increase in inhibitory phosphorylation of its catalytic subunit PP2Ac at Tyr307," and build a mechanism in which the environmental toxin β -N-methylamino-L-alanine acts through metabotropic glutamate receptor 5 and Src to dissociate the catalytic subunit and expose it to phosphorylation at that residue. Every step of that mechanism downstream of the activity measurement is read through the phospho-tyrosine-307 antibody, which binds unphosphorylated catalytic subunit with equal affinity and is eighty to ninety per cent blocked by methylation at leucine 309 (Frohner and colleagues, 2020a; Mazhar and colleagues, 2020). The activity measurement itself is independent of that reagent and stands; the mechanism attached to it does not. We cite the paper for its buffer and for nothing else, and we note that a rise in its antibody signal is most parsimoniously read as a fall in methylation at leucine 309 — which would make it, unintentionally, a methylation result in a second tauopathy.

What follows for every number in this volume. No activity figure quoted anywhere here is a measurement of the phosphatase's total inactivation in the living brain. Each is a measurement

of what remained after the tissue died, after the buffer was added, and — where the immunoprecipitation route was used — after the antibody discarded the holoenzymes. The direction of all three artefacts is the same, and it is toward the null.

X. Two Relocations

This is the section the volume was written for, and the observation in it did not exist before this scan. It is made by putting two results on the same page, and neither result is new.

The first departure. Under hydrogen peroxide, the phosphatase methylesterase PME-1 moves out of the nucleus and into the cytoplasm. The movement is CRM1-dependent nuclear export, established by its abolition with leptomycin B in a human embryonic kidney line stably expressing a green-fluorescent-protein-tagged methylesterase; the demethylated, phosphorylated form of the catalytic subunit redistributes with it; N-acetylcysteine reverses the nuclear-to-cytoplasmic ratio of both; and — the point on which everything turns — **the protein expression of the methylesterase remained unchanged** (Tang and colleagues, 2018a). The systems are human dermal fibroblasts and human embryonic kidney cells.

The finding is therefore not that oxidative stress makes more of the enzyme. It is that oxidative stress moves the enzyme it already has. That is a different mechanism with different properties: it is fast, it does not require transcription, it is reversible by a reductant, and it changes the *ratio* of methylesterase to methyltransferase in each compartment without changing either total.

The second departure. In Alzheimer brain, the endogenous inhibitor SET moves out of the nucleus and into the cytoplasm. The ratio of neurons with immunonegative nuclei to neurons with immunopositive nuclei is more than sixfold greater in Alzheimer than control tissue; nuclear signal falls; full-length cytosolic protein falls; and an approximately twenty-kilodalton cytosolic fragment appears in most Alzheimer and few control cases (Tanimukai, Grundke-Iqbal and Iqbal, 2005). The mechanism is proteolysis: asparaginyl endopeptidase cuts the inhibitor at asparagine 175 (Basurto-Islas and colleagues, 2013), and that residue sits *inside* the bipartite nuclear localisation signal spanning residues 168 to 181. The cut does not remove the signal from one fragment and hand it to the other; it bisects it, and since cytoplasmic retention in the original mutagenesis required disruption of both basic motifs, neither half of a bisected signal should suffice for import. **The cleavage and the redistribution are not two events; they are one.**

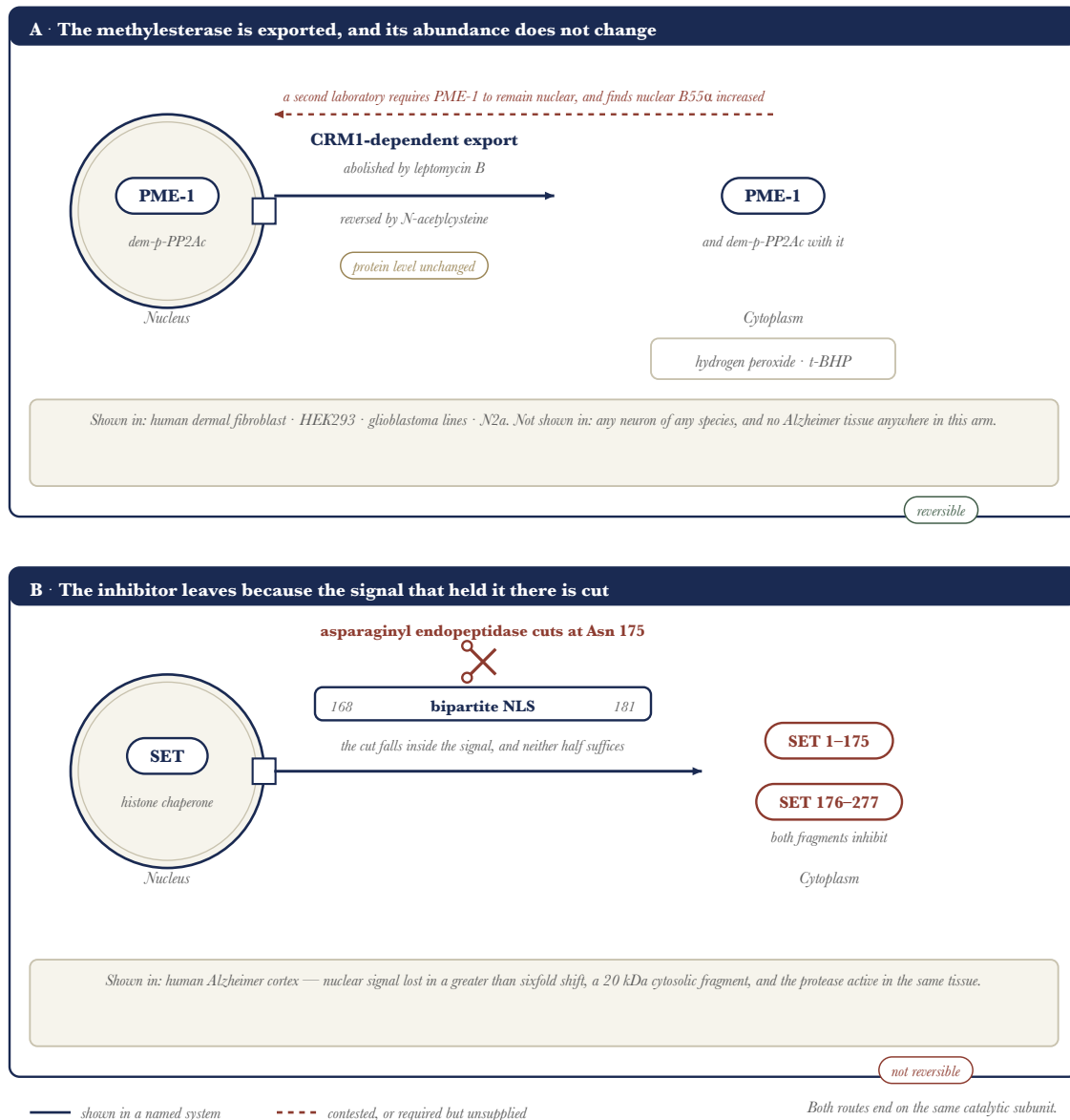


Figure 1. Two regulators of the same catalytic subunit leave the nucleus in the same disease. The methyltransferase is exported intact and its abundance does not change; the inhibitor leaves because the signal that held it there has been cut in half. The mechanisms are unrelated, and so is the quality of the evidence: the upper panel has no neuron and no Alzheimer tissue in it, and a second laboratory requires the methyltransferase to remain nuclear for its own effect, which is drawn here as the competing arrow it is. The lower panel is human Alzheimer cortex. Only one of the two departures is irreversible.

Why the juxtaposition is worth making. Two proteins that regulate the same catalytic subunit, in the same disease, both leave the same compartment. One is the enzyme that removes the methyl ester governing which holoenzyme the cell can build; the other is the inhibitor that binds the

catalytic subunit directly. Both arrive in the cytoplasm, which is where tau is. On the face of it this is a convergence, and a tidier volume would call it one.

It is not a convergence, and the differences are more instructive than the similarity. Four of them, and each is load-bearing.

The mechanisms are unrelated. One is regulated transport through a nuclear pore by an export receptor; the other is destruction of a targeting sequence by a protease. There is no shared upstream, no shared machinery, and no reason a cell doing one should do the other.

One is reversible and one is not. Nuclear export can be reversed — by leptomycin B experimentally, by N-acetylcysteine in the published system, and presumably by the cell itself when the oxidant is withdrawn. Proteolysis cannot be reversed by anything; a cell cannot re-ligate a cleaved protein. *The Four Locks* identified this asymmetry as the reason the proteolytic lock is the one that makes the lesion permanent, and it applies here unchanged.

The evidence is of entirely different quality, and the asymmetry is exact. The inhibitor's departure is a human Alzheimer observation in autopsy cortex with a regional control. The methylesterase's departure is a fibroblast and a kidney line. **No study has measured the methylesterase's compartment in human Alzheimer tissue**, and a structural parallel between a human disease observation and a cell-line observation is a parallel between two different kinds of claim.

Worse for the parallel: the one thing that *has* been measured about the methylesterase in human Alzheimer brain is the other variable. Its **abundance is increased** in Alzheimer and progressive supranuclear palsy frontal cortex, alongside decreased methyltransferase and a seventy-five per cent fall in the methylation ratio (Park and colleagues, 2018). So in the tissue that matters, the methylesterase changes in the way the redox literature says it does *not* change, and the variable the redox literature reports has never been looked at. The juxtaposition of this section is between a measured human relocation and a hypothesised one.

And the compartmental direction is contested. This is the finding that most complicates the section, and it did not appear in the scan that commissioned it. A second laboratory, working in glioblastoma lines, reports that oxidative stress increases the binding of the methylesterase to the B55 α -containing holoenzyme and increases demethylation of B55 α -bound catalytic subunit — and that **the nuclear localisation of the methylesterase is required** for the effect, along with its methylesterase function and its phosphatase-binding capacity. In the same work, oxidative stress *increased* the nuclear localisation of the B55 α subunit (Guffens, Derua and Janssens, 2023). One laboratory has the methylesterase leaving the nucleus under oxidant; the other requires it to stay there, and finds its substrate arriving. Both used hydrogen peroxide. Neither used a neuron.

We do not resolve this. We record that the two are compatible only under conditions nobody has specified — different oxidant doses, different durations, different cell lineages, a transient export

followed by return — and that a volume proposing nuclear export as a disease mechanism must reckon with a study requiring nuclear retention for the same enzyme's effect on the same holoenzyme.

What the juxtaposition actually yields, which is not a mechanism but a redirection. If the two departures do not share a mechanism, share only one compartment, and differ in reversibility and in evidence, then what is left in common is a *consequence*: in both cases, **the nucleus loses a regulator of this phosphatase.** The entire Alzheimer literature on both proteins is written about what they do when they arrive in the cytoplasm — the inhibitor gags the phosphatase, the methylesterase demethylates the catalytic subunit — and almost nothing is written about what stops happening in the nucleus when they leave.

That is a real gap and it is specific. The inhibitor's nuclear job is substantial: it is a histone chaperone and the core subunit of the inhibitor-of-acetyltransferases complex, suppressing p300, CREB-binding protein and PCAF acetyltransferase activity by masking histones from the enzymes rather than by inhibiting them. A neuron that exports its histone-masking protein has a chromatin lesion as well as a phosphatase lesion, and the corpus has a volume on the chromatin side of this disease that does not currently connect to this one. The methylesterase's nuclear job is, on the second laboratory's evidence, to set the methylation state of the nuclear B55 α -bound pool — so a neuron that exports it has a *nuclear* phosphatase that is differently methylated, governing nuclear substrates nobody in this literature has measured.

So the section's finding is stated as a question rather than a mechanism, which is what the evidence supports. The two relocations are not one event seen twice. They are two unrelated events with one shared consequence, and the shared consequence is on the wrong side of the nuclear envelope from where everyone has been looking. **Nobody has measured the nuclear pool of this phosphatase, its methylation state, or its holoenzyme composition, in a human Alzheimer neuron.** Section XV states the measurement that would distinguish a genuine two-compartment lesion from a coincidence of direction, and Section XVI weighs it against the volume's primary experiment.

One thing the section must not do, and it is a live risk in this corpus. The companion volume *The Silenced Eraser* reports, from the inflammasome study's figures, that inflammasome-bearing animals carry **elevated levels** of the methylesterase. The redox literature reports **unchanged levels** and altered localisation. These are not in conflict and neither corrects the other: they are two different routes to the same enzyme, one acting on abundance through an inflammatory signal, the other on compartment through a chemical one. A cell subject to both would have more of the enzyme *and* have it in the wrong compartment. Stated that way the two are additive, which is a prediction — and it is the only place in this volume where the speck arm and the

phosphatase arm make contact without passing through the neuronal reactive-species step that Section VI graded Weak.

XI. The Methyl Ester

The second route is the one whose chemistry is best understood and whose disease evidence is, in the form the spine requires, absent. This section separates what is known about the methyl ester from what is known about oxidative stress acting on it.

The lock itself, restated from the companion volume. Leucine carboxyl methyltransferase 1 transfers a methyl group from S-adenosylmethionine to the carboxyl terminus of leucine 309 on the catalytic subunit; the methylesterase PME-1 removes it. The modification is a reversible ester, so the equilibrium is set by the relative activity of the two enzymes and by the cell's methyl donor economy. Methylation is required for assembly of B55-family holoenzymes — of which B α is the member that matters for tau — while B' and B'' assembly carries no absolute requirement (Longin and colleagues, 2007). The methyl group is therefore not a global switch on catalysis; it is a **selector** determining which holoenzyme the cell assembles. And the methylesterase does more than de-esterify: its structure bound to the A–C core shows that it binds the active site and inactivates the enzyme by evicting the catalytic manganese ions.

The human disease evidence is good, and it is not about oxidative stress. Methyltransferase protein expression and catalytic subunit methylation are quantitatively decreased in Alzheimer-affected brain regions; the regional loss of methyltransferase immunoreactivity parallels the severity of tau pathology rather than amyloid plaque burden; and the methyltransferase is abundant in cortical neurons in normal and non-Alzheimer demented controls (Sontag and colleagues, 2004b). Set beside the holoenzyme deficit measured in the same tissue class (Sontag and colleagues, 2004a) and the finding that the B α *transcript* is not reduced, the three compose a coherent post-transcriptional account: the cell makes normal amounts of B α messenger RNA, fails to methylate enough catalytic subunit to assemble and stabilise the holoenzyme, and loses the holoenzyme protein.

The afferent established for that account is **one-carbon metabolism** — folate, homocysteine, the ratio of S-adenosylmethionine to S-adenosylhomocysteine — with gain- and loss-of-function arms in cells and in mice, and a dietary manipulation that works in vivo. It is not oxidative stress. Any claim that the disease's demethylation is redox-driven is a claim in competition with a better-evidenced one, and this volume does not advance it.

What the redox literature adds, and in which systems. Four studies, and the reader should hold the cell type in mind for each.

Hydrogen peroxide induces demethylation of the catalytic subunit and inhibits mechanistic-target-of-rapamycin complex 1 signalling; both a methylesterase inhibitor and overexpression of the methyltransferase prevent the demethylation and the downstream suppression, and methyltransferase overexpression rescues cell viability and mitochondrial membrane potential. **Human embryonic kidney cells** (Tang and colleagues, 2018b).

Hydrogen peroxide relocates the methylesterase from nucleus to cytoplasm by CRM1-dependent export with abundance unchanged, carrying the demethylated phosphorylated catalytic subunit with it, reversibly with N-acetylcysteine. **Human dermal fibroblasts and human embryonic kidney cells** (Tang and colleagues, 2018a).

Oxidative stress increases methylesterase binding to the B55 α holoenzyme and demethylation of B55 α -bound catalytic subunit, requiring the methylesterase's methylesterase function, its phosphatase-binding capacity and its nuclear localisation; the consequence in that system is sensitisation to oxidative-stress-induced death through MAPKAPK2 and RIPK1. **Glioblastoma lines U87MG and U251MG** (Guffens, Derua and Janssens, 2023).

And manganese exposure, which raises oxidative stress, lowers catalytic subunit methylation and deranges autophagy through mechanistic-target-of-rapamycin complex 1 and ULK1; both the methylesterase inhibitor ABL-127 and methyltransferase overexpression restore methylation and ameliorate the autophagy defect and the cytotoxicity. **Neuro-2a cells** (Xu and colleagues, 2021).

Three qualifications, stated rather than softened. The last of these is the closest approach to a neuron in the whole arm, and Neuro-2a is a mouse neuroblastoma line, not a neuron; its oxidative stress is a *correlate* of manganese exposure rather than an isolated variable, so the chain from reactive species to demethylation is not separated from manganese's several other effects. The third is the only one that identifies the holoenzyme — and it identifies B55 α , which is the right one for tau, which makes it the most valuable result in the arm and the one furthest from a brain. And none of the four measures tau.

There is no neuron and no Alzheimer tissue in the redox half of this arm. But there is Alzheimer tissue in the arm itself, and it is better than we expected when this volume was commissioned. The methylesterase *has* been measured in human Alzheimer brain, once, and the result is important enough to state in full.

In frontal cortex from Alzheimer and progressive supranuclear palsy cases against controls, by immunohistochemistry and by immunoblot, methylated catalytic subunit was reduced and demethylated catalytic subunit increased, **with no change in total catalytic subunit and no change in the B α subunit**, giving a fall in the methylated-to-demethylated ratio of approximately seventy-five per cent in Alzheimer disease and sixty-three per cent in progressive supranuclear palsy;

and this was accompanied by **a decrease in the methyltransferase and an increase in the methylesterase in both diseases** (Park and colleagues, 2018).

Four things follow, and they pull in different directions.

The lesion is confirmed and quantified in human tissue, and it is large. A seventy-five per cent fall in the methylation ratio is a bigger number than any activity deficit in this literature, and it was obtained with reagents that report methylation directly rather than through the discredited carboxy-terminal phospho-antibody.

It is not specific to this disease. Progressive supranuclear palsy shows the same lesion at nearly the same magnitude. That is a useful control against an agonal artefact and a caution against treating demethylation as an Alzheimer mechanism rather than a tauopathy mechanism.

It resolves an apparent conflict with the holoenzyme data rather than creating one. Park and colleagues find total B α unchanged; Sontag and colleagues find the assembled AB α C holoenzyme reduced in the same disease. Those are different measurements — a subunit and a trimer — and their combination is exactly the post-transcriptional assembly failure the methylation account predicts: the cell has the subunit, cannot methylate enough catalytic subunit to recruit it, and loses the assembled enzyme while the free subunit pool stays put.

And it is the wrong kind of change for this volume's premise. The human finding is that the methylesterase is **increased in abundance**. The redox finding is that the methylesterase is **unchanged in abundance and moved between compartments**. The human tissue therefore supports the mechanism the companion volume *The Silenced Eraser* proposes — an inflammatory route acting on the enzyme's level — and not the mechanism this volume was written to test. Nobody has measured the methylesterase's *compartment* in human Alzheimer tissue at all, and Section X's structural argument depends on somebody doing so.

Re-reading the discredited literature as methylation data, which recovers something. Here the withdrawal of phospho-tyrosine 307 pays a dividend rather than costing one. The commercial antibodies raised against that site are blocked eighty to ninety per cent by methylation at leucine 309 (Mazhar and colleagues, 2020), and are not specific for the phosphorylation they are named for (Frohner and colleagues, 2020a). It follows that **a reported rise in "phospho-tyrosine-307" signal is most parsimoniously a reported fall in leucine-309 methylation** — which is precisely the lesion of this section, measured by accident.

That conversion is worth something and it is worth stating what it costs. It is directionally sound and quantitatively useless: the antibodies are also sensitive to phosphorylation at threonine 304, at least one of them prefers the phosphopeptide somewhat, and one cross-reacts with phosphotyrosine generally, so the signal is a composite of at least three modifications with unknown weights. Nothing quantitative may be extracted. What may be extracted is a direction, and the direction agrees with

the reagents that do not have this problem. The Guam parkinsonism-dementia study of Section IX becomes, on this reading, a report of reduced phosphatase activity accompanied by reduced methylation in a second tauopathy — which is a more interesting result than the one it published.

Grade: Moderate for the chemistry, Absent for the disease. The mechanism by which oxidative stress demethylates the catalytic subunit is well established across four studies and two continents, with pharmacological and genetic rescue in both directions. It has been established in a fibroblast, a kidney line, two glioblastoma lines and a mouse neuroblastoma line. It has never been shown in a neuron, never in brain tissue of any species, and never in this disease.

XII. CIP2A

The third route has the strongest human evidence in the volume and the weakest connection to the volume's premise. It is also entirely new to this corpus: before this volume, CIP2A had zero occurrences across every file, and that absence was a real gap rather than a correct omission.

What it is, and where it comes from. CIP2A — Cancerous Inhibitor of Protein Phosphatase 2A, the product of *KIAA1524* — was described as an endogenous inhibitor of this phosphatase with oncogenic activity. It interacts directly with the transcription factor c-Myc, inhibits phosphatase activity toward c-Myc serine 62, and thereby prevents c-Myc's proteolytic degradation; it promotes anchorage-independent growth and tumour formation, transforms human cells on overexpression, and is overexpressed in head and neck squamous cell carcinoma and colon cancer (Junttila and colleagues, 2007). Every subsequent statement about this protein originates in cancer biology, and this volume labels each transfer to the neuron as a transfer.

What it binds, and this is the section's decisive result. CIP2A forms homodimers. The crystal structure of an amino-terminal fragment spanning residues 1 to 560, solved at three-ångström resolution, established the dimerisation interface, which was then confirmed by structure-based mutational analysis. And **the CIP2A dimer interacts with the phosphatase regulatory subunits B56 α and B56 γ** , binding them through a conserved amino-terminal region, with dimerisation promoting that binding; inhibiting either the dimerisation or the expression of B56 α and B56 γ destabilises CIP2A itself (Wang and colleagues, 2017). The mechanism was independently corroborated in a different disease: oncogenic KRAS upregulates CIP2A, and does so in order to sequester B56 α away from the active holoenzyme in a CIP2A-dependent manner (Tinsley and colleagues, 2024).

Set that against Section III and the consequence is immediate. The subunit that makes this enzyme a tau phosphatase is B α , which is B55 α , the product of *PPP2R2A* — a member of the B55 family. B56 is the B' family, structurally unrelated, differently regulated, and, on the assembly biochemistry, not even subject to the same methylation requirement at leucine 309.

CIP2A's structurally defined target is therefore **not the tau phosphatase**. It is a different holoenzyme with a different address book and a different substrate list.

And the founding mechanism says the same thing in functional terms, which is easy to miss because of how the protein is named. Junttila and colleagues did not report that CIP2A inhibits the phosphatase; they reported that it inhibits phosphatase activity *toward c-Myc serine 62*. CIP2A is a **substrate-selective** inhibitor, and the substrate it selects is a B56 substrate. The transfer from oncology to the neuron has imported a selective inhibitor and applied it to a substrate belonging to the other subunit family.

The human Alzheimer evidence, which is nonetheless the best in this volume. CIP2A is overexpressed in human Alzheimer brain. CIP2A-mediated phosphatase inhibition drives tau and amyloid precursor protein hyperphosphorylation and increases β -cleavage and amyloid- β production; increased CIP2A leads to tau mislocalisation to dendrites and spines and to synaptic degeneration; adeno-associated-virus delivery of CIP2A into mouse hippocampus induces cognitive deficits and impairs long-term potentiation; and increased CIP2A expression in Alzheimer brain **results from increased amyloid- β** , closing a feed-forward loop (Shentu and colleagues, 2018). Independently, checkpoint kinase 1 activity and CIP2A levels are elevated in human Alzheimer brains, in APP/PS1 mice and in amyloid- β -treated primary neurons; checkpoint-kinase-1 overexpression induces CIP2A upregulation, phosphatase inhibition, tau and precursor hyperphosphorylation, synaptic impairment and memory deficit; and the checkpoint-kinase-1 inhibitor GDC0575 raises phosphatase activity, lowers tau phosphorylation, inhibits amyloid- β overproduction, and reverses cognitive deficits and neuron loss in APP/PS1 mice (Hu and colleagues, 2022).

That is a human observation, a sufficiency demonstration in vivo, a necessity demonstration by pharmacology, and a named upstream. No other arm in this volume has all four. **The arm is Strong, and it is the only Strong arm here.**

Its upstream is not redox, and this volume will not pretend otherwise. The demonstrated chain is amyloid- β to DNA damage to checkpoint kinase 1 to CIP2A. It was established in the Alzheimer setting (Hu and colleagues, 2022) and extended in a chronic-restraint-stress model, where corticotropin-releasing-factor treatment and restraint stress raise DNA damage, which activates checkpoint kinase 1, which upregulates CIP2A, producing tau hyperphosphorylation, amyloid- β overproduction, synaptic impairment and cognitive deficit (Wang and colleagues, 2024).

The redox bridge, however, is drawn — and this volume was commissioned on the belief that it was not. The scoping pass that produced this volume recorded that the oxidative-damage bridge is undrawn in every one of these papers and instructed that the corpus must not assert it. That instruction was based on the abstracts. In the full text of the

checkpoint-kinase-1 study there is an experiment that draws it: primary cortical neurons were treated with hydrogen peroxide for two hours, and the authors report that this induced **checkpoint kinase 1 activation, CIP2A overexpression, suppression of phosphatase activity and tau hyperphosphorylation, all of which checkpoint-kinase-1 inhibition reversed**, with checkpoint-kinase-1 serine-345 phosphorylation correlating with increased DNA damage and increased CIP2A (Hu and colleagues, 2022). *We carry this from the published full text rather than the abstract and have not seen the figures; readers relying on it should check them.*

That is a considerably better result than the volume expected, and it must be credited before it is qualified. It is in a **primary neuron**, not a line. It runs reactive species to checkpoint kinase 1 to CIP2A to the phosphatase to tau, in one system, with a pharmacological reversal. It is the only place in this entire volume where an oxidant, a neuron and this phosphatase appear in the same experiment.

Now the qualifications, and they are substantial. The oxidant is a two-hour bolus of hydrogen peroxide at a concentration far above anything a neuron experiences in a slowly progressive disease; the paper does not measure oxidised bases, so the specifically *oxidative-DNA-damage* link is inferred within the paper as it is outside it; and no upstream kinase is identified — the authors leave open whether the ataxia-telangiectasia-mutated kinase, the ataxia-telangiectasia-and-Rad3-related kinase or DNA-dependent protein kinase phosphorylates checkpoint kinase 1 here. The companion intervention in the chronic-stress model is **vitamin C**, a promiscuous agent administered against "DNA damage" rather than a defined species, in a restraint-stress rather than an amyloid model (Wang and colleagues, 2024).

And there is a specific objection that this literature has not answered, which the volume raises here. Checkpoint kinase 1's activation through the ataxia-telangiectasia-and-Rad3-related kinase is canonically a *replication-stress* response, and a post-mitotic neuron does not replicate. That is not fatal by itself — a non-canonical, replication-independent route from hydrogen peroxide to this kinase has been demonstrated in arrested cells — but two further facts make the neuronal case harder rather than easier. In neurons, inhibiting the ataxia-telangiectasia-and-Rad3-related kinase does not rescue death and may worsen it, and deleting it from post-mitotic neurons enhances neuronal firing without compromising development, by a mechanism the authors state is independent of damage-response signalling (Kirtay and colleagues, 2021). And in Alzheimer neurons the better-established damage kinase, ataxia-telangiectasia mutated, is *reduced* rather than raised. A route that requires a neuron to run a checkpoint it does not otherwise run needs the checkpoint demonstrated, not assumed.

The same objection applies to CIP2A itself, and more sharply. CIP2A's best-characterised molecular functions are cell-cycle functions: it is enriched at spindle poles on mitotic entry, it participates in centrosome separation, and — decisively — its role in the

DNA-damage response is explicitly mitosis-gated. CIP2A is actively exported from the nucleus during interphase and gains access to chromatin only when the nuclear envelope breaks down at the onset of mitosis, where it acts in a complex with TOPBP1. **A neuron never breaks down its nuclear envelope.** So a model in which DNA damage in a post-mitotic neuron acts through CIP2A must either propose a non-mitotic CIP2A function or accept that CIP2A here is downstream of checkpoint kinase 1 as a transcriptional target rather than as a participant in repair. The published chain — checkpoint kinase 1 raising CIP2A *levels* — is in fact consistent with the second reading, and that is the reading this volume adopts. It is worth stating because the phrase "DNA damage response" imports a machinery that a neuron cannot run.

What else CIP2A binds, and the caution it carries. The only systematic interactome of this protein was performed in T helper 17 cells and recovered, besides the known regulatory subunits, the catalytic subunits of the phosphatase, and — among the top novel interactors — the regulatory and catalytic subunits of protein phosphatase 1, namely PPP1R18, PPP1R12A and PPP1CA (Khan and colleagues, 2020). Two things follow. If CIP2A contacts the catalytic subunit directly in some settings, then a route to holoenzymes other than B56 is not excluded, and the structural objection above becomes a strong prior rather than a proof. And if CIP2A also engages protein phosphatase 1, then "CIP2A inhibits PP2A" understates its reach — which matters here because protein phosphatase 1 performs approximately eleven per cent of tau dephosphorylation in human brain and was, in the rat vicinal-thiol survey of Section VIII, the phosphatase that *was* recovered as redox-active. That is a coincidence and is recorded as one. It is also a lymphocyte interactome, which is a transfer of a transfer.

The therapeutics, with one correction. Two compounds that lower CIP2A have been tested against Alzheimer phenotypes, and they are not equivalent. Polyphyllin I reduced CIP2A, activated the phosphatase, decreased tau and precursor phosphatase-site phosphorylation and amyloid- β in CIP2A-overexpressing human embryonic kidney cells and in **primary neurons**, restored synaptic protein levels, and — in **3 $\times$ Tg-AD mice** — decreased CIP2A, reactivated the phosphatase, prevented hyperphosphorylation and amyloid- β overproduction and rescued cognitive impairment (Zhou and colleagues, 2020). Genistein reduced CIP2A expression and restored phosphatase activity, lowering precursor phosphorylation at threonine 668, amyloid- β production and tau phosphorylation at serines 396 and 404 — in **HEK293-T cells co-transfected with CIP2A and either the precursor or tau, and in nothing else** (Shentu and colleagues, 2019). The scan that commissioned this volume described both as rescuing Alzheimer phenotypes in models. One does. The other was tested only in a transfected human embryonic kidney line, and the corpus should not repeat the stronger claim.

A reagent and abundance caution, entered because this corpus has been burned once. Following the rule of Section IV, the clone is named. Nearly the whole neuronal and Alzheimer CIP2A literature uses one polyclonal reagent, **ABclonal A12267**, in primary neurons, in

mouse brain and in human Alzheimer cortex; the Parkinson's work adds Cell Signaling 2038T and 14805S and Santa Cruz sc-80662. We have not located a knockout-validated immunohistochemical characterisation of any of them, and the Human Protein Atlas's own single CIP2A antibody is scored *Uncertain* for immunoblot, with the tissue page recording low consistency between antibody staining and transcript.

Three abundance facts belong beside that. CIP2A transcript in human brain is low and regionally unremarkable — of the order of one to four transcripts per million across cortical regions, an order of magnitude below lymphocyte and testis, with the cerebellum the highest brain region. The field's own imaging reports CIP2A expressed at considerable level in astrocytes relative to neurons in primary co-culture, and the same group has a separate paper in which CIP2A drives **astrogliosis** to produce synaptic degeneration. And every human Alzheimer CIP2A measurement is a western blot on bulk cortex; **we could not locate a single CIP2A immunohistochemistry on human Alzheimer brain sections**, which means the human observation has never been resolved to a cell type.

That last point is the one to carry forward. A protein measured in bulk cortex, whose transcript is low in neurons and whose protein the same laboratory reports as higher in astrocytes, may be reporting a glial change. The volume's human anchor is therefore less neuronal than it looks, and Section XVI's public-data experiment is the cheap way to find out.

A further caution about the literature's shape. The Alzheimer CIP2A body of work is almost entirely the output of one group and its collaborators. That is not a criticism — it is the same fact recorded for the disulfide arm in Section VIII — but a claim graded *Strong* on human tissue should note that the human tissue has been examined by one laboratory tradition.

Grade: Strong in human tissue for the observation and the sufficiency; Absent for the redox trigger; and structurally misdirected for the tau target. The arm holds up better than any other in this volume and supports a different conclusion from the one it was recruited for.

XIII. Do the Three Routes Converge?

The question can now be put precisely. Three routes to one phosphatase were proposed to share one upstream event. Do they?

They do not converge on a target, and this is the volume's principal finding.

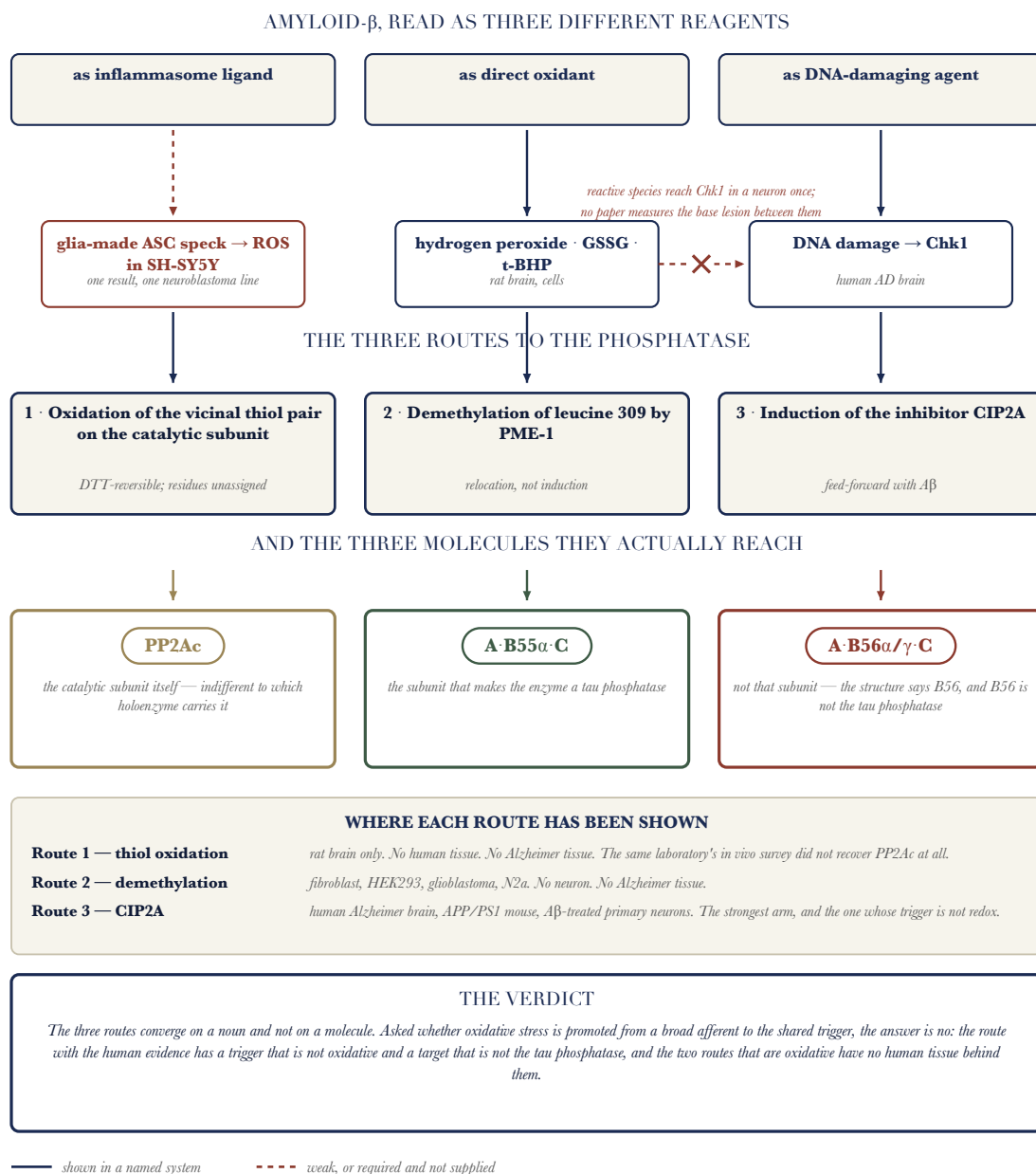


Figure 2. Each route is drawn to the molecular object it has actually been shown to act on, rather than to the word PP2A. Oxidation of the vicinal thiol pair acts on the catalytic subunit and is indifferent to which holoenzyme carries it; loss of the methyl ester at leucine 309 acts specifically on recruitment of the B55 α subunit, which is the subunit that makes the enzyme a tau phosphatase; and the inhibitor whose human evidence is strongest binds B56 α and B56 γ , which are not that subunit. The upstream row records what each route's demonstrated afferent is. Only one route has human Alzheimer tissue behind it, and it is the one whose trigger is not oxidative and whose target is not the tau phosphatase.

Route one, oxidation of the vicinal thiol pair, acts on the **catalytic subunit**. The thiols are on the C subunit; the inhibition is of catalysis, reversed by a reductant; and it is indifferent to which regulatory subunit the subunit is wearing. Whatever it does, it does to every holoenzyme at once.

Route two, demethylation at leucine 309, acts on **B55 α recruitment**. Methylation is required for B55-family assembly and not for B' or B'' assembly (Longin and colleagues, 2007), and the one redox study that identifies the holoenzyme identifies B55 α (Guffens, Derua and Janssens, 2023). This route is exquisitely specific, and it is specific to the right thing: B55 α is the tau phosphatase.

Route three, induction of CIP2A, acts on **B56 α and B56 γ** — on a crystal structure, a mutational analysis and an independent corroboration in a second disease (Wang and colleagues, 2017; Tinsley and colleagues, 2024). B56 is not B55. On the structural evidence, the route with the human Alzheimer observation behind it acts on a holoenzyme that is not the tau phosphatase.

Three routes, three molecular objects, one noun. The convergence in the list is a convergence of vocabulary. It exists because "PP2A" names a family of roughly ninety-six holoenzymes and because the assay in general use cannot distinguish them — an immunoprecipitation with a carboxy-terminus-directed monoclonal, which preferentially recovers non-methylated catalytic subunit and fails to bring down B- or B'-containing trimers (Frohner and colleagues, 2020b), reports all three of these lesions as "decreased PP2A activity" and can report none of them as what it is.

This is the same subtraction the companion volume performed, and the repetition is informative. *The Four Locks* found that SET binds the catalytic subunit and pulls down neither the scaffold nor a B subunit, and concluded that the inhibitor lock probably does not act on the same molecular object as the three assembly locks. This volume finds that the other endogenous inhibitor in the disease literature binds B56 α and B56 γ . **Both of the endogenous protein inhibitors that the Alzheimer literature invokes fail to localise onto the AB α C holoenzyme**, by two independent pieces of structural evidence, obtained twenty years apart in two unrelated laboratories. A pattern that appears twice by different methods is worth stating as a pattern: the disease literature's inhibitors of "PP2A" are inhibitors of pools of this enzyme that nobody has shown to be the tau-directed pool.

Nor do they converge on a trigger, and the failure is sharper than last time. Section VI graded the speck-to-neuron arm Weak on one result in one neuroblastoma line. Section VIII found the thiol arm rat-only, unrecovered by the survey that looked for it in living brain, and stable across the lifespan. Section XI found the demethylation arm to have no neuron and no disease tissue in it. And Section XII found the one arm with human Alzheimer evidence to have a demonstrated upstream — DNA damage and checkpoint kinase 1 — that is not oxidative.

So the arrangement is the opposite of the one the spine proposed. **The two routes that are genuinely oxidative have no human tissue behind them; the route with human tissue behind it is not oxidative.** Oxidative stress is not promoted from a broad afferent to the shared trigger. It remains what the companion volume found it to be: a broad afferent with one direct arm onto this enzyme's own thiols, and that arm is now graded lower than it was.

What survives, stated fairly. Three things survive and they are not nothing.

The *feed-forward loop* is real and human: amyloid- β raises CIP2A, CIP2A inhibits the phosphatase, the inhibited phosphatase permits increased β -cleavage and amyloid- β production (Shentu and colleagues, 2018). That loop does not require oxidative stress and does not require the speck. It is the best-evidenced route from amyloid- β to this phosphatase in the literature, and this volume's contribution to it is the warning that its target subunit is wrong for tau.

The *coincidence of compartment* is real: two regulators leave the nucleus in this disease, and nobody has measured the nuclear pool.

And the *invisibility* is real: an assembly lesion is discarded by the antibody, a redox lesion is undone by the buffer, and both were undone before any number was published.

The architecture, restated. The companion volume described a coincidence structure — an enzyme with an unusual number of independent regulatory surfaces in a cell subject to an unusual number of simultaneous insults. This volume adds a dimension to that description. The surfaces are not only independent in their *afferents*; they are attached to **different holoenzymes**, which means the lesions are not even acting on the same population of enzyme molecules. What looked like one enzyme being switched off four ways, and then three more, is better described as a *family* of enzymes being switched off in different members, by different insults, and then summed by an assay that cannot tell the members apart.

That is a weaker claim than the list implies and a considerably more useful one, because it makes a prediction the convergent model does not. If these lesions act on different holoenzyme pools, then the total "PP2A activity" deficit is a sum of small deficits in distinguishable pools, no single intervention will normalise it, and the therapeutically relevant question is not *how much* phosphatase activity is lost but *which holoenzyme*. Nobody has measured the holoenzyme composition of this enzyme in Alzheimer tissue beyond the single AB α C measurement of 2004.

XIV. The Validity Ledger

Each joint is graded by what was measured, in which species, in which tissue and with which reagent — not by how often the claim has been repeated. Six tiers are used: *Established*, *Strong*,

Moderate, Weak, Absent for a claim whose evidence does not exist, and *Rejected as stated*. Two entries are *Corrections* to the companion volumes.

Established (human, structural, inherited) — the subunit that makes this enzyme a tau phosphatase is B55 α , and methylation at leucine 309 governs its recruitment. Direct binding to tau at a domain indistinguishable from its microtubule-binding domain (Sontag and colleagues, 1999); crystal structure and reconstitution showing that B α markedly facilitates dephosphorylation of phospho-tau (Xu and colleagues, 2008); B55-family assembly requires the methyl ester while B' and B'' assembly does not (Longin and colleagues, 2007). *This entry is the instrument the volume uses and is not itself at issue.*

Established (human) — methyltransferase protein and methylated catalytic subunit are reduced in Alzheimer-affected regions, in a regional pattern paralleling tau pathology rather than amyloid burden (Sontag and colleagues, 2004b). *Afferent is one-carbon metabolism, not oxidative stress.*

Established (human) — the endogenous inhibitor SET is transcriptionally increased, translocated from nucleus to cytoplasm, and cleaved to an approximately twenty-kilodalton fragment in Alzheimer cortex, with no change in cerebellum (Tanimukai, Grundke-Iqbal and Iqbal, 2005). *Note: this study makes no phosphatase activity measurement of its own; its opening statement that phosphatase activity is compromised in this disease is a citation to other work. Any review citing it as an activity paper has miscited it.*

Established (mouse, genetic, with antibody control) — microglia-derived ASC specks bind amyloid- β and cross-seed it, and inflammasome loss reduces tau hyperphosphorylation and aggregation by regulating tau kinases and phosphatases (Venegas and colleagues, 2017; Ising and colleagues, 2019). *The specificity of the phosphatase limb — that the regulator concerned is PME-1 — is carried from the companion volume at second hand and is not verified here.*

Strong (human tissue, plus sufficiency and pharmacological necessity in vivo) — CIP2A is overexpressed in Alzheimer brain, is sufficient to produce tau and precursor hyperphosphorylation, synaptopathy and memory deficit, and is raised by amyloid- β in a feed-forward loop (Shentu and colleagues, 2018; Hu and colleagues, 2022). *The single strongest arm in this volume. Caveat: antibody clones unverified here.*

Strong (structural, oncology, independently corroborated) — CIP2A binds the B56 α and B56 γ regulatory subunits, not B55 α . Homodimer; crystal structure of residues 1 to 560 at three ångström; structure-based mutational analysis; binding through a conserved amino-terminal region; destabilisation of CIP2A on loss of B56 α/γ (Wang and colleagues, 2017); sequestration of B56 α from the active holoenzyme in a CIP2A-dependent manner in a second disease (Tinsley and colleagues, 2024). *This is the entry that forces the volume's negative answer. It is an*

oncology result and is labelled as a transfer; what it establishes is a strong prior against CIP2A acting on the tau phosphatase, not a proof.

Strong (human AD tissue, mouse, primary neuron) — the demonstrated upstream of CIP2A in Alzheimer disease is amyloid- β to DNA damage to checkpoint kinase 1 (Hu and colleagues, 2022; Wang and colleagues, 2024). *Not oxidative stress.*

Moderate (biochemical, rat brain extract, one laboratory) — the phosphatase is directly and reversibly inhibited by oxidation of a vicinal thiol pair on the catalytic subunit. Hydrogen peroxide and glutathione disulfide inhibit the major constitutive rat-brain phosphatase, which is this enzyme, by a dithiothreitol-reversible mechanism (Foley, Armstrong and Kupchak, 2004); thiol-reactive agents and phenylarsine oxide produce reversible inhibition with up to sevenfold recovery of specific activity on reduction (Foley and Kintner, 2005; Foley and colleagues, 2007); a fraction of catalytic subunit carries disulfides after anti-artefact homogenisation (Foley and colleagues, 2011). *Caveats: one laboratory; no human tissue; no Alzheimer tissue; residues unassigned; and see the two entries below.* **Downgraded from Strong — see Corrections.**

Moderate (cells, four systems, both directions) — oxidative stress demethylates the catalytic subunit, and the mechanism is pharmacologically and genetically reversible. Hydrogen peroxide induces demethylation, prevented by a methylesterase inhibitor or methyltransferase overexpression (Tang and colleagues, 2018b); oxidative stress increases methylesterase binding to the B55 α holoenzyme and demethylation of B55 α -bound catalytic subunit (Guffens, Derua and Janssens, 2023); manganese lowers methylation in a mouse neuroblastoma line, rescued by ABL-127 and by methyltransferase overexpression (Xu and colleagues, 2021). *Systems: human dermal fibroblast, HEK293, U87MG, U251MG, Neuro-2a. No neuron; no brain tissue; no disease tissue; no tau measured.*

Moderate (cells, contested direction) — the methylesterase changes compartment under oxidative stress, with its abundance unchanged. CRM1-dependent nuclear export abolished by leptomycin B, demethylated-phosphorylated catalytic subunit redistributing with it, reversed by N-acetylcysteine (Tang and colleagues, 2018a). *Contested: a second laboratory requires the methylesterase's nuclear localisation for its effect and reports increased nuclear B55 α under the same oxidant (Guffens, Derua and Janssens, 2023). Both used hydrogen peroxide; neither used a neuron.*

Moderate (rat, in vivo, direction established and magnitude unknown) — global ischaemia produces a transient reductive shift in brain protein vicinal thiol redox couples (Foley and colleagues, 2024). *Consequence, if it holds in human tissue: the manner of death partly reduces the lesion before collection. Never tested in human brain and never tested at a post-mortem interval.*

Weak (one cell line, one study, provenance noted) — an ASC speck raises reactive oxygen species in a neuron. Glia-produced ASC specks induce reactive oxygen species in SH-SY5Y cells, reported within a study of ethanol binge-drinking in APP/PS1 mice (Brezani and colleagues, 2025). *SH-SY5Y is a neuroblastoma line, not a neuron. No mechanism of neuronal entry is demonstrated.*

Moderate (primary neuron, one experiment, supraphysiological oxidant) — reactive oxygen species reach CIP2A through checkpoint kinase 1 in a neuron. Hydrogen peroxide applied to primary cortical neurons for two hours induced checkpoint-kinase-1 activation, CIP2A overexpression, suppression of phosphatase activity and tau hyperphosphorylation, all reversed by checkpoint-kinase-1 inhibition, with serine-345 phosphorylation correlating with DNA damage and CIP2A (Hu and colleagues, 2022, full text). Supported by vitamin C suppressing the pathway in a chronic-restraint-stress model (Wang and colleagues, 2024). *This is the only experiment in the volume containing an oxidant, a neuron and this phosphatase together, and the commissioning scan reported it as absent because it is not in the abstract. Caveats: a two-hour bolus far above physiological concentration; no oxidised bases measured; no upstream kinase identified; carried from full text without sight of the figures.*

Weak (contested by neuronal physiology) — a post-mitotic neuron runs the checkpoint this route requires. Deleting the ataxia-telangiectasia-and-Rad3-related kinase from post-mitotic neurons enhances firing without compromising development, by a mechanism its authors state is independent of damage-response signalling (Kirtay and colleagues, 2021); inhibiting it in neurons does not rescue death and may worsen it; and the better-established neuronal damage kinase, ataxia-telangiectasia mutated, is *reduced* in Alzheimer neurons. *Do not import "DNA damage response" machinery into a neuron without demonstrating it.*

Weak (mechanistically obstructed) — CIP2A participates in a neuronal DNA-damage response. CIP2A's damage function is mitosis-gated: it is exported from the nucleus in interphase and reaches chromatin only on nuclear-envelope breakdown, acting with TOPBP1. A neuron never breaks down its nuclear envelope. *The published chain — checkpoint kinase 1 raising CIP2A levels — is consistent with CIP2A as a transcriptional output rather than a repair participant, and this volume adopts that reading.*

Absent — cell-type resolution for the human CIP2A observation. Every human Alzheimer CIP2A measurement is an immunoblot on bulk cortex; we could not locate a single CIP2A immunohistochemistry on human Alzheimer sections. Transcript in human brain is low, the field's own co-culture imaging reports CIP2A higher in astrocytes than neurons, and the same group has a separate paper in which CIP2A acts through astrogliosis. *The volume's strongest human anchor may not be neuronal.*

Absent — S-glutathionylation of the phosphatase catalytic subunit. Not demonstrated in any system located, and specifically found low and unchanged in the rat brain tissue in which vicinal-thiol disulfides were found to vary (Foley and colleagues, 2014; Foley, Katchur and Gillespie, 2016). *Claims that this enzyme is regulated by glutathionylation should be withdrawn or restated.*

Absent — residue-level assignment of the reversible vicinal thiol pair on the catalytic subunit. The thiols are defined operationally, as vicinal and arsenical-reactive. Residue-level assignment exists only for irreversible electrophile adducts: four cysteines in the catalytic subunit alkylated by the inhibitory of two probes and not by the non-inhibitory one, with a homology model relating adducts to inhibition (Codreanu and colleagues, 2006), in a human embryonic kidney expression system. *We have not verified those four residue numbers against the full text.*

Established (human, two tauopathies) — the methylesterase is increased and the methyltransferase decreased in Alzheimer frontal cortex, with the methylation ratio down approximately seventy-five per cent and total catalytic subunit and B α subunit unchanged. The same pattern at similar magnitude in progressive supranuclear palsy (Park and colleagues, 2018). *Two consequences: the human change in the methylesterase is one of abundance, not compartment, which supports the inflammatory route rather than the redox one; and unchanged total B α beside reduced assembled AB α C (Sontag and colleagues, 2004a) is what post-transcriptional assembly failure looks like.*

Absent — any measurement of the phosphatase's redox state in human Alzheimer brain, or of the methylesterase's compartment. Searched and not located. Every result in the disulfide arm is rat; every result in the methylesterase *localisation* arm is a cell line. More broadly, no thiol-redox assay — biotin switch, polyethylene-glycol switch, differential alkylation, OxICAT or equivalent — has been performed on any protein phosphatase in human Alzheimer brain. Reversible-cysteine proteomics of human Alzheimer brain exists only in the S-nitrosylation sub-genre, and no phosphatase appears in its published protein lists.

Moderate (structural inference, not measurement) — the reversible vicinal pair is cysteine 266 and cysteine 269. The catalytic subunit carries ten cysteines; 266 and 269 form a CXXC motif; in the solved core-enzyme and holoenzyme structures their sulfurs lie about four and a half to five and a half ångström apart and **no other pair in the protein is within nine ångström**. Cysteine 269 is the covalent target of fostriecin and phoslactomycin and is required for microcystin inhibition. *The redox-switch assignment itself is made in a review citing papers that name no residue; it is an inference and is entered as one. A separation of four and a half ångström is not a disulfide and requires rearrangement to become one.*

Rejected — cysteines 50 and 55 are a second vicinal thiol pair. They are five residues apart in sequence and more than fifteen ångström apart in the solved holoenzyme. The claim appears in the review literature and should be withdrawn.

Absent — the co-measurement. No study has measured PME-1, CIP2A, LCMT-1, Ba and the redox state of the catalytic subunit in the same human Alzheimer tissue, let alone in the same neurons.

Absent — internalisation of an extracellular ASC speck by a neuron. Searched and not located in any system. Microglial and astrocytic uptake is established.

Absent — the bridge from oxidative DNA base damage specifically to checkpoint kinase 1 in a neuron. Reactive species reach checkpoint kinase 1 in a neuron (entry above), but no study measures 8-oxo-guanine or any defined base lesion alongside the kinase in a neuron, and no study identifies the sensor kinase. The one paper linking 8-oxo-guanine specifically to this checkpoint did so through replication stress in dividing cancer cells, which is the thing a neuron cannot supply. *Do not assert the base-damage link; the reactive-species link may now be asserted with the caveats above.*

Established (human, independent laboratories, chemically rigorous) — oxidative DNA base damage is present in Alzheimer brain and is present already in mild cognitive impairment. Threefold elevation of 8-hydroxy-2'-deoxyguanosine in mitochondrial DNA (Mecocci, MacGarvey and Beal, 1994); multiple oxidised bases elevated by gas chromatography–mass spectrometry with **no correlation to tangle or plaque counts** (Gabbita, Lovell and Markesbery, 1998); the same damage present at the earliest detectable disease stage (Wang, Markesbery and Lovell, 2006); focal γ H2AX double-strand-break labelling raised in neurons from mild cognitive impairment onward (Shanbhag and colleagues, 2019). *This supplies the substrate the CIP2A arm needs. It does not supply the link.*

Absent — any measurement of the phosphatase activator PTPA in human Alzheimer brain. Searched and not located. An assertion in the cell-culture literature that PTPA is decreased in Alzheimer brain could not be traced to any primary human measurement and should not be repeated.

Reported as null, and now with a published source — genetic association of the phosphatase system with Alzheimer risk. *The Four Locks* reported this as searched and unpublished. There is a published negative: across 729 Alzheimer cases and 670 controls, variation in *PPP2CA*, *PPP2R2A*, *ANP32A*, *LCMT1*, *PPME1* and *PLIN1* showed no difference in genotypic, allelic or haplotypic distribution (Vázquez-Higuera and colleagues, 2011). **The corpus entry should be changed from "searched; none published" to "tested and negative."**

Rejected as stated — the three routes converge on one molecule. They act on the catalytic subunit, on B55 α recruitment and on B56 α/γ respectively. The convergence is in the noun.

Rejected as stated — oxidative stress is the shared trigger disabling this phosphatase in Alzheimer disease. The two oxidative routes have no human tissue behind them; the route with human tissue has a non-oxidative demonstrated upstream.

Rejected as stated — two CIP2A-lowering compounds rescue Alzheimer phenotypes in models. One does, in 3×Tg-AD mice and primary neurons (Zhou and colleagues, 2020). The other was tested in transfected HEK293-T cells and nowhere else (Shentu and colleagues, 2019).

Correction to *The Four Locks* — the vicinal-thiol entry is downgraded from Strong to Moderate. That volume graded the claim **Strong (biochemical, brain tissue)** in its prose ledger and **Strong / Rat brain tissue** in its summary table, on three Foley papers. Six further papers from the same laboratory exist. Two of them bear directly on the grade: the in vivo survey of vicinal-thiol oxidation in healthy rat brain recovered the sodium/potassium adenosine triphosphatase catalytic subunit, tubulins, glyceraldehyde-3-phosphate dehydrogenase and protein phosphatase 1, and did **not** recover protein phosphatase 2A (Foley, Katchur and Gillespie, 2016); and the lifespan survey found vicinal-thiol-linked redox states **stable with age** (Foley and colleagues, 2024). **The grade should read Moderate**, with those two caveats attached, in both places.

Correction to *The Four Locks* — the buffer claim is restated as conditional. That volume asserted that every human activity measurement was made in buffer containing reducing agent, at ten millimolar β-mercaptoethanol. The recipe is verified verbatim in two papers from that laboratory (Basurto-Islas and colleagues, 2013; Arif and colleagues, 2014), one of which measured activity in rat slices and the other in Guam parkinsonism-dementia rather than Alzheimer disease. In the four papers carrying the canonical Alzheimer numbers the buffer is **not recoverable** from anything we can access. The claim is a well-motivated prediction about four methods sections, not an established fact, and should be stated that way.

Entered as an omission from the corpus — a negative human result. Measuring the same phosphatases in Alzheimer temporal cortex, one study found no significant activity difference between Alzheimer and control for any phosphatase examined, and found phosphatase 2A *protein* increased in the postsynaptosomal fraction (Pei and colleagues, 1998). Three of its authors are authors of the papers reporting the deficit. Neither companion volume carries it. **The Established grade that both volumes assign to the human activity deficit should carry this failed replication as an explicit caveat.**

Summary of grades

Claim	Tier	Basis
B55α is what makes the enzyme a tau phosphatase; Leu309 methylation governs its recruitment	Established	Structure + reconstitution

Claim	Tier	Basis
LCMT-1 and methylated C reduced in AD-affected regions, tracking tau not plaques	Established	Human
SET increased, translocated and cleaved in AD cortex, not cerebellum	Established	Human
ASC specks bind and cross-seed A β ; inflammasome loss reduces tau pathology	Established	Mouse, genetic + antibody
CIP2A overexpressed in human AD brain; sufficient in vivo; raised by A β	Strong	Human + mouse + neuron
CIP2A binds B56 α and B56 γ , not B55 α	Strong	Crystal structure + mutagenesis
CIP2A's AD upstream is A β $\rightarrow$ DNA damage $\rightarrow$ Chk1	Strong	Human + mouse + neuron
PME-1 up, LCMT-1 down, methylation ratio down ~75% in AD and PSP cortex	Established	Human, two tauopathies
Oxidative DNA base damage in AD brain, present already at MCI	Established	Human, GC/MS, two laboratories
PP2A reversibly inhibited by vicinal thiol oxidation	Moderate (was Strong)	Rat brain extract, one laboratory
Oxidative stress demethylates PP2Ac	Moderate	Fibroblast, HEK293, GBM, N2a
PME-1 changes compartment under oxidant, abundance unchanged	Moderate	Cells; direction contested
The reversible vicinal pair is Cys266/Cys269	Moderate	Structural inference, not measurement
Global ischaemia gives a transient reductive shift in brain vicinal thiols	Moderate	Rat, in vivo
ROS $\rightarrow$ Chk1 $\rightarrow$ CIP2A $\rightarrow$ PP2A $\rightarrow$ tau, in a neuron	Moderate	Primary neuron, one experiment, 200 μ M H ₂ O ₂
ASC speck raises ROS in a neuron	Weak	One result, SH-SY5Y, ethanol study
A post-mitotic neuron runs the ATR/Chk1 checkpoint this route needs	Weak	Contested by neuronal ATR physiology
CIP2A participates in a neuronal DNA-damage response	Weak	Its damage role is mitosis-gated

Claim	Tier	Basis
S-glutathionylation of PP2Ac	Absent	Not demonstrated; excluded in rat brain
Residue <i>assignment</i> of the reversible vicinal pair	Absent	Inference only; never measured
PP2Ac redox state in human AD brain; PME-1 compartment in human AD	Absent	Never measured
Any thiol-redox assay on any phosphatase in human AD brain	Absent	Never performed
Cell-type resolution for the human CIP2A observation	Absent	Bulk blots only; may be glial
PTPA measured in human AD brain	Absent	Never measured
PME-1, CIP2A, LCMT-1, Ba and PP2Ac redox in one AD tissue	Absent	Never measured
Neuronal internalisation of an extracellular ASC speck	Absent	Never demonstrated
Oxidative <i>base damage</i> → Chk1 in a neuron	Absent	Never drawn
Cys50/Cys55 are a second vicinal pair	Rejected	15.4 Å apart in the structure
The three routes converge on one molecule	Rejected	Three different holoenzymes
Oxidative stress is the shared trigger	Rejected	Oxidative arms lack human tissue
Two CIP2A-lowering compounds rescue AD phenotypes	Rejected as stated	One is HEK293-T only
<i>The Four Locks</i> : vicinal-thiol entry graded Strong	Correction	Downgrade to Moderate
<i>The Four Locks</i> : buffer claim stated unconditionally	Correction	Restate as conditional
<i>The Four Locks</i> : PP2A-pathway genetics "none published"	Correction	Tested and negative (Vázquez-Higuera 2011)
Human PP2A activity deficit in AD	Caveat added	Failed replication (Pei 1998)

XV. Predictions and Falsification

A mechanism earns its place by naming what would destroy it. The predictions below are ordered from cheapest to most demanding, and each ends with the result that would refute the claim it tests. Several of them can refute this volume's own central arguments, which is the point.

1. The buffer claim is discharged by reading four methods sections. Obtain the published Materials and Methods of Gong 1993, Gong 1995, Liu 2005 and Sontag 2004a, and record the homogenisation and assay buffer composition. The prediction is that each contains a thiol reducing agent, most probably β -mercaptoethanol at approximately ten millimolar in homogenisation and twenty millimolar in the assay, consistent with the verified recipe of the same laboratory. *Falsified if any of the four omitted reducing agent and still reported a deficit — which would show that the deficit does not require the redox component to have been reduced away, and would remove the motivation for prediction 2.* This costs a library card and an hour, and until it is done the argument of Section IX is conditional.

2. The phosphatase deficit in Alzheimer brain is larger than reported, because part of it is a disulfide. Assay tau-directed phosphatase activity in paired aliquots of the same Alzheimer and control homogenates, one prepared with and one without reducing agent, with the non-reduced arm assayed before and after dithiothreitol add-back. The difference between Alzheimer and control will be *larger* in the non-reduced condition, and dithiothreitol will restore proportionally more activity in Alzheimer than in control extract. *Falsified if reductant restores equal proportions in both, which would show the redox pool is not differentially oxidised in disease and would close the redox arm of this volume.*

3. The catalytic subunit carries more oxidised vicinal thiols in Alzheimer than in control cortex. This is the volume's primary experiment and it is specified in Section XVI. *Falsified if the redox-shift distribution of the catalytic subunit is indistinguishable between Alzheimer and control while the positive-control proteins behave as the rat surveys predict — a clean negative that would end the disulfide arm as a disease mechanism rather than leaving it undecided.*

4. CIP2A does not inhibit the tau phosphatase, because it is not bound to it. Immunoprecipitate CIP2A from human Alzheimer and control cortex and from CIP2A-overexpressing primary neurons, and blot the precipitate for B55 α , B56 α , B56 γ , the scaffolding subunit and the catalytic subunit. On the structural evidence, B56 α and B56 γ will be recovered and B55 α will not. Reciprocally, immunoprecipitate B55 α and ask whether CIP2A co-precipitates. *Falsified if CIP2A recovers B55 α from neuronal or Alzheimer tissue, which would show that the neuron does something the oncology structure does not predict and would restore the convergence this volume denies.* **This is the single most consequential experiment named here,** because the volume's negative answer rests on a structure obtained in cancer cells and the transfer to the neuron has never been checked.

5. If CIP2A does not bind B55 α , then its tau effect is indirect, and the indirection is findable. In the same system, ask whether CIP2A's effect on tau phosphorylation survives knockdown of B56 α and B56 γ , and whether it is accompanied by changes in the phosphorylation of known B56 substrates — including glycogen-synthase-kinase-3 β , whose activity state is itself a tau determinant. The prediction is that CIP2A raises tau phosphorylation by deranging a kinase through a B56 holoenzyme rather than by removing an eraser through a B55 one. *Falsified if tau phosphorylation rises with no change in any B56 substrate and no change in kinase activity, which would require a direct route to the tau phosphatase that has not been described.* The companion volume found precisely this pattern for a different B-subunit knockout, in which tau pathology arose from deranged kinases rather than from failed erasure, so the mechanism has a precedent in this enzyme family.

6. The demethylation route runs through B55 α and is therefore the only oxidative route aimed at tau. In a neuron — a primary cortical culture, not a line — apply a defined oxidant at a dose that does not kill, and measure methylation of the catalytic subunit, the amount of B55 α recoverable in complex with it, and tau phosphorylation at phosphatase-favourable sites. The prediction is that demethylation, loss of B55 α from the complex, and a rise in tau phosphorylation occur together and in that order. *Falsified if demethylation occurs without loss of B55 α from the complex, which would sever the only oxidative route that reaches the tau phosphatase and would leave this volume with no oxidative route to tau at all.*

7. The compartmental contradiction resolves into a dose or a time course. Repeat the methylesterase localisation experiment across oxidant dose and duration in one cell type, scoring nuclear-to-cytoplasmic ratio of the methylesterase and of B55 α together. The prediction is that export and retention are the same phenomenon at different points — a transient export at higher dose, retention at lower — and that the two laboratories sampled different points. *Falsified if the direction is invariant across dose and time within one cell type, which would mean the discrepancy is between lineages and that neither result may be generalised to a neuron.*

8. The nucleus loses this phosphatase's regulation before the cytoplasm gains it. Quantify, in human Alzheimer and control cortex staged by conformational tau epitopes, the nuclear and cytoplasmic pools of the catalytic subunit, its methylation state, B55 α , PME-1 and SET, cell by cell. The prediction of Section X is that nuclear depletion of the regulators is detectable in neurons bearing conformational but not fibrillar tau. *Falsified if nuclear and cytoplasmic changes appear together or if the nuclear change follows the fibrillar one, which would make the two relocations a consequence and would remove the redirection this volume proposes.*

9. The oxidative bridge to checkpoint kinase 1 survives at a physiological oxidant dose, and runs through a base lesion. The bridge exists at two hundred micromolar hydrogen peroxide for two hours. Repeat it across a dose–time series down to concentrations a neuron plausibly experiences, and measure the missing variable: oxidised guanine bases, alongside

checkpoint-kinase-1 serine-345 phosphorylation and CIP2A, in amyloid- β -treated primary neurons, with a defined antioxidant rather than vitamin C. *Falsified if the kinase and CIP2A respond only at bolus concentrations, or if amyloid- β raises them with no rise in base damage, either of which would make the published result a peroxide-toxicity phenomenon rather than a disease mechanism.*

9a. And the checkpoint the route requires is actually run. In the same neurons, establish which sensor kinase phosphorylates checkpoint kinase 1 — ataxia-telangiectasia mutated, ataxia-telangiectasia-and-Rad3-related, or DNA-dependent protein kinase — by selective inhibition and epistasis. *Falsified as a damage-response mechanism if none of the three is required, which would mean the oxidant reaches the kinase by a route that has nothing to do with DNA and would remove the CIP2A arm's only claim on the word "damage."*

10. The manner of death reduces the lesion. In rodent brain, compare vicinal-thiol redox state of the catalytic subunit between rapid freezing and a simulated agonal and post-mortem interval of several hours at body and then ambient temperature. The prediction is that measured oxidation falls with post-mortem interval. *Falsified if oxidation is stable or rises across the interval, which would remove the second arm of Section IX and would also, usefully, make human autopsy tissue a fair substrate for prediction 3.* This is the experiment that should be done *before* prediction 3, because it determines whether prediction 3 can succeed at all.

11. Speck abundance is not sufficient, and the cerebellum says so. In the 5xFAD cerebellum, where ASC specks are more abundant than in cortex and plaques are absent, measure the phosphatase's methylation state, B55 α content and tau phosphorylation. The prediction, on this volume's negative answer, is that none of them differs from wild-type cerebellum despite the speck excess. *Falsified if the speck-rich cerebellum shows a phosphatase lesion without a plaque burden, which would rehabilitate the speck arm and make it independent of amyloid deposition — a stronger result than the one originally proposed.*

12. The two endogenous inhibitors act on pools the assay cannot distinguish. Fractionate Alzheimer and control cortex by holoenzyme composition rather than by total catalytic subunit — immunoprecipitating B55 α , B56 α and the free catalytic pool separately — and assay each against tau and against a B56 substrate. The prediction is that the tau-directed deficit localises to the B55 α fraction and the CIP2A-associated deficit to the B56 fraction, and that a total-catalytic-subunit assay sums them into a number that describes neither. *Falsified if the deficit is proportionally equal across fractions, which would mean the lesion is on the catalytic subunit after all and would rehabilitate the redox route as the general mechanism.*

XVI. The Experiment

Three candidates were considered and one is recommended. The recommendation is not the cheapest of the three, and it is the only one that no arm of this literature has performed in human tissue.

The recommendation: a redox-shift blot of the catalytic subunit from human Alzheimer and control cortex.

Tissue. Frozen frontal or temporal cortex from neuropathologically confirmed Alzheimer cases and age-matched controls, with cerebellum from the same brains as the internal negative region — with the caution of Section VI that the cerebellum is a weaker control for an oxidative question than for a tangle-linked one, since it is not exempt from lipid peroxidation. Post-mortem interval must be recorded for every case and treated as a covariate rather than a nuisance, for the reason prediction 10 gives. Agonal state should be recorded where the brain bank holds it, which is seldom.

Homogenisation, and this is the whole experiment. No reducing agent of any kind. The tissue is disrupted directly into a buffer containing a large excess of a membrane-permeant thiol-alkylating agent — *N*-ethylmaleimide or iodoacetamide — so that every thiol reduced at the moment of disruption is capped irreversibly and cannot re-oxidise during handling. This is the trapping step that the rat work established (Foley and colleagues, 2014), and without it the experiment measures handling rather than disease. Free alkylating agent is then removed, existing disulfides are reduced with **tris(2-carboxyethyl)phosphine** — a phosphine, not a thiol, for the reason established when the affinity chemistry was improved (Foley and colleagues, 2010) — and the newly liberated thiols are labelled with polyethylene-glycol maleimide, which adds a defined mass per thiol.

Readout. Immunoblot for the catalytic subunit. Each thiol that was oxidised in vivo now carries a polyethylene-glycol adduct and the band shifts upward by a discrete, countable increment; each thiol that was reduced in vivo was capped and does not shift. The distribution of the catalytic subunit across shifted species is a direct readout of its in vivo thiol oxidation state, resolved to the number of oxidised cysteines. A biotin-switch variant, substituting a biotinylated maleimide and a streptavidin capture, gives a yes-or-no answer more cheaply and should be run first as a feasibility check.

The confirmatory arm, which finally assigns the residues. Excise the shifted species and identify the modified cysteines by mass spectrometry, asking specifically whether the oxidised pair is cysteine 266 and cysteine 269 — the only vicinal pair in the protein, and the pair a review has inferred without anyone measuring it. Two outcomes are informative and both are publishable: the inference is confirmed, and a thirty-year operational definition becomes a structural one; or the oxidised cysteines are elsewhere, and the arsenical-reactive "vicinal" thiols of the brain-extract literature are not the structurally obvious pair, which would be more interesting still. Note the digestion hazard the field has already met once at this terminus — the carboxy-terminal tryptic

peptides of this subunit ionise poorly — so an endoproteinase Lys-C digest should be run alongside trypsin.

Controls, and the rat surveys have already named them. Proteins whose vicinal thiols are known to be oxidised in brain under physiological redox variation — peroxiredoxins 1 and 2 as the peroxide sensors, the sodium/potassium adenosine triphosphatase catalytic subunit, tubulins, glyceraldehyde-3-phosphate dehydrogenase, creatine kinase B and α -enolase — serve as positive controls for the chemistry, and **protein phosphatase 1 serves as the internal comparator that was recovered where protein phosphatase 2A was not** (Foley, Katchur and Gillespie, 2016; Foley and colleagues, 2024). Neuron-specific enolase is the reported negative. If the controls shift and the catalytic subunit does not, the arm is dead and the experiment has succeeded.

Run beside it. Paired aliquots of the same homogenates assayed for tau-directed phosphatase activity with and without reducing agent, and with dithiothreitol added back to the non-reduced arm, exactly as prediction 2 specifies. The activity arm and the blot arm answer the same question by different means, and agreement between them is worth more than either alone. The activity assay must not use a carboxy-terminus-directed monoclonal immunoprecipitation; it must be a direct assay in extract, for the reason Section III gives.

Why this one. No study anywhere has measured the redox state of this enzyme in human tissue, let alone diseased human tissue. Every result in the arm is rat. The single most consequential gap in the volume is therefore also the cheapest to close, it requires no new reagent, its positive controls are already published, and — decisively — **it can return a clean negative**. An experiment that can only confirm is not worth running.

The first alternative, and it is arguably more important. The co-immunoprecipitation of prediction 4: does CIP2A bind B55 α in a neuron? This volume's negative answer rests on a crystal structure obtained in cancer cells, and a structure is a strong prior and not a proof. If CIP2A recovers B55 α from Alzheimer cortex, Section XIII is wrong and the convergence is restored. The experiment is a day's work for a laboratory that already has the tissue and the antibodies, and it tests the volume's central claim rather than its central hope. **A reader with one experiment to spend and a preference for settling an argument should spend it here.** The redox-shift blot is recommended above it only because the redox question is the one nobody can answer at all at present, whereas this one merely has not been asked.

The second alternative, which should be done because it is nearly free. Interrogate the published single-nucleus and single-soma datasets — the entorhinal and frontal atlas, and the tangle-bearing soma census — for *PPP2CA*, *PPP2R2A*, *PPP2R5A*, *PPP2R5C*, *LCMT1*, *PPME1*, *KIAA1524*, *CHEK1* and *SET*, comparing vulnerable against resistant excitatory subtypes and tangle-bearing against tangle-free somas. The companion volume named this experiment for its own gene set and nobody has performed it; this volume adds four genes to the list. Two specific

predictions attach: that *KIAA1524* will prove to be expressed at low level in neurons and no higher in vulnerable subtypes, which would qualify the human CIP2A observation severely; and that *PPME1* will not distinguish vulnerable from resistant subtypes, which would locate the methylesterase arm downstream of whatever determines vulnerability. The cost is compute, and the honest caveat is that both genes may sit below the detection floor of single-nucleus sequencing, in which case the result is uninformative rather than negative.

And the cheapest of all, which should be done first. Run CIP2A immunohistochemistry on human Alzheimer and control sections, double-labelled for a neuronal and an astrocytic marker. Every human CIP2A measurement in this literature is a bulk immunoblot; the field's own co-culture imaging puts CIP2A higher in astrocytes than neurons; and the same group has a paper in which CIP2A acts through astrogliosis. If the human signal is glial, the strongest arm in this volume is an arm about astrocytes, and the whole argument moves one cell type across. That is a staining experiment on tissue that already exists.

What a rational programme would look like. Establish the post-mortem stability of the measurement in rodent (prediction 10). Read four methods sections (prediction 1). Then run the redox-shift blot and the reducing-agent-free activity re-assay on the same homogenates, and the CIP2A co-immunoprecipitation on the same tissue. That is four experiments, one of which is a library errand and one of which is a mouse study, and together they would settle whether this volume's negative answer is right — or replace it with a better one.

XVII. Coda The Word and the Molecule

There is a version of this volume that was easy to write and would have been wrong. It begins with amyloid- β , assembles a speck, licenses a burst of reactive species, oxidises a thiol pair on a catalytic subunit, drives a methylesterase out of a nucleus, induces an inhibitor, and arrives at a silenced phosphatase and a hyperphosphorylated tau — one trigger, three routes, one enzyme. Every arrow in that sentence has been drawn somewhere. The sentence is still wrong, and the reason it is wrong is not that any of the arrows is fabricated. It is that the enzyme at the end of them is not one enzyme.

Three literatures converged on a name. The redox biochemists were studying a catalytic subunit in rat brain extract. The methylation biochemists were studying the recruitment of one regulatory subunit out of twenty-three. The cancer biologists were studying an inhibitor that binds two members of a different subunit family altogether. Each of them wrote "PP2A," because that is what the protein is called, and the assay that the disease literature used to join them could not have told them apart if they had asked it to: an antibody that prefers the demethylated subunit and leaves the regulatory subunits in the supernatant returns a single number, and a single number cannot report that three different holoenzymes were inhibited in three different fractions of the same

homogenate. The convergence was real at the level of the word and absent at the level of the molecule, and no amount of care about the upstream would have caught it, because the error was downstream.

That is the same shape as the error the companion volume found, and finding it twice in one enzyme is the part worth keeping. There, a modification that was never demonstrated became the standard account of how this phosphatase is switched off, sustained for twenty-eight years by antibodies that were reporting a methyl group two residues away. Here, three genuine mechanisms are summed into one claim by a noun that names ninety-six molecules. Both errors are errors of resolution rather than of evidence. Both were invisible from inside any one of the contributing literatures. And both were found the same way — not by a new experiment, but by putting papers that do not cite each other on the same page and asking whether they are about the same object.

The third finding is quieter and is about what a measurement can survive. If part of this enzyme's inactivation in the living brain is a disulfide, then it has been undone twice before anybody counted it: once by the ischaemia of dying, which a rat study says shifts brain thiols toward reduction, and once by the reducing agent in a homogenisation buffer, which is not a decision anybody makes but a line inherited from whoever trained you. A lesion that is erased by the manner of death and again by the first step of the protocol is not a lesion anyone has looked at and failed to find. It is a lesion nobody has been in a position to see. Whether it exists is an open question, and the reason it is still open after thirty years is that the method arrived before the question did.

The list was right about the cast. Amyloid- β is upstream, the speck is real, the thiols are oxidisable, the methyl ester is lost in this disease, and CIP2A is raised in Alzheimer brain and had no entry in this corpus until now. What the list got wrong is what lists always get wrong: it assumed that things which arrive at the same word arrive at the same place.

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Every entry below was verified against PubMed for journal, year, volume, issue, pages, title and author list before it was written, and carries a digital object identifier. Where a claim in the text rests on material we could read only at abstract level, or on a full text whose figures we did not see, that limitation is stated inline at the point of use rather than here. Four citations inherited from the companion volumes were found to carry transposed PubMed identifiers — the journal, volume and page strings were correct in every case — and the corrected records are the ones used here.

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*Companion volumes: **The Four Locks** (the same enzyme approached from inside the neuron — four described ways to switch it off, and what survives when they are asked to converge); **The Silenced Eraser** (the eraser approached from outside the neuron — the ungoverned microglion, the inflammasome, and the route to PME-1 by abundance rather than by compartment); **The Single Cut** (one protease, two substrates, and the two-compartment lesion that follows from cleaving SET). This volume is the sequel to the first: it takes the one arm of oxidative stress that survived that adjudication and asks whether it can be promoted from a broad afferent to the shared trigger. It cannot, and the reason is a crystal structure.*