Sequential Failure of Proteostasis Mechanisms

Summary

Proteome remodeling across mini-pools of Alzheimer's disease neurons reveals a sequential breakdown of proteostasis: an early, biphasic instability of the ubiquitin–proteasome system is followed later by a mechanistically distinct disruption of lysosomal acidification. V-ATPase subunits initially show compensatory upregulation in tau-positive neurons but collapse beyond a misfolded-tau (MC1) threshold, consistent with a failed-compensation model and a staged failure of protein quality control arms.

Background

Tau aggregation and misfolding impose a heavy proteostasis burden on neurons, which normally balance protein turnover through the ubiquitin–proteasome system (UPS) and the autophagy–lysosome pathway (ALP). As tau misfolds and accumulates, cells invoke compensatory responses to triage damaged proteins, but these mechanisms can fail under chronic load, leading to proteome-wide remodeling, metabolic compromise, and ultimately neuronal dysfunction or death. Here, proteomic measurements from mini-pools of 10 neurons, stratified by tau status and ordered by precomputed pseudotime or misfolded-tau burden (MC1), are leveraged to dissect the temporal ordering and mechanistic coupling between UPS and lysosomal acidification failure.

Results & Discussion

A covariate-controlled differential expression analysis (age, PMI, and PatientID) across 5,853 proteins (BH-FDR) identified 2,115 proteins (36.14%) significantly altered between tau-positive and tau-negative neurons, with a predominance of downregulation (1,288 down; 827 up; mean effect $= -0.28$ log2) and large mean absolute effect size (0.584 log2), indicating broad proteome remodeling in tau-positive cells [r1-1]. Notably, the autophagy receptor SQSTM1 was the most upregulated protein ($+3.41$ log2), consistent with activation of selective autophagy, whereas marked decreases were observed for SLC2A1 (glucose transporter), LMNA (prelamin-A/C), and PODXL (-2.14 to -2.57 log2), with collagens (COL1A1, COL1A2, COL6A2) showing the largest declines (> -4.0 log2) [r1-1]. The robustness of these differences is supported by narrow confidence intervals and very low FDR-adjusted p-values (e.g., 10^{-13} to 10^{-17} for top hits), together pointing to a state of proteome-wide stress in tau-positive neurons with selective upregulation of autophagic adapters amidst widespread loss of structural and metabolic components [r1-1].

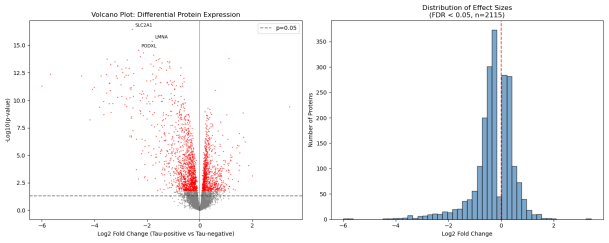

Figure 3: Tau-positive neurons exhibit widespread proteome remodeling dominated by protein downregulation. (A) Volcano plot of differential protein expression between tau-positive and tau-negative neurons, with significantly altered proteins highlighted in red (FDR < 0.05). (B) Histogram showing the distribution of log2 fold changes for the 2,115 significantly altered proteins. The left-skewed distribution of effect sizes indicates a broad loss of protein components in neurons with tau pathology. (Source: [Trajectory r1-1])

To resolve temporal dynamics, we constructed a Proteasome Score by averaging log2 expression across 17 core subunits (PSMA1–7, PSMB1–10) and modeled its trajectory along pseudotime. Segmented regression significantly outperformed a single linear fit ($F = 6.68$, $p = 0.0031$; $\Delta R^2 = 0.2493$; AIC -110.8 vs -102.2), revealing a breakpoint at pseudotime 0.3724 where an early uptrend (slope $+1.33$) reverses to decline (slope -0.92) [r1-24]. This inverted-U (compensate-then-fail) behavior indicates that UPS components are initially mobilized but cross an early tipping point where capacity erodes, consistent with an early failure of proteasomal protein quality control despite modest linear correlation overall ($r = -0.067$, $p = 0.67$) [r1-24]. Functionally, this early inflection posits a phase where misfolded tau and associated damaged proteins overwhelm proteasomal throughput, necessitating increased reliance on autophagic mechanisms—an interpretation reinforced by the strong SQSTM1 upregulation in tau-positive neurons [r1-1, r1-24].

The lysosomal acidification arm, quantified via V-ATPase subunits and a V-ATPase Score, reveals a delayed but decisive failure. Individual V-ATPase subunits (ATP6V1A/B2/C1/H) were significantly elevated in tau-positive versus tau-negative neurons (mean difference +0.239 log2; all $p < 0.022$), indicative of early or ongoing compensation [r1-19]. However, within tau-positive cells, these subunits correlated strongly and negatively with MC1 (mean $r = -0.723$; range -0.581 to -0.791; all $p < 0.005$), with expression highest at low MC1 and progressively reduced as misfolded-tau burden rose, supporting a failed-compensation model under increasing pathology [r1-19]. A segmented analysis of the V-ATPase Score against MC1 located a critical threshold at MC1 = 2.831 where a near-stable phase (slope -0.0029) transitions into a steep decline (slope -0.4255), and the segmented model improved fit over a single linear model ($F = 4.359$, $p = 0.0286$; R^2 0.712 vs 0.573), pinpointing a collapse threshold for lysosomal acidification under high tau burden [r1-80]. When ordered by pseudotime, the V-ATPase system also showed significant biphasic behavior, but with a later breakpoint (0.654) than the proteasome (difference 0.282 pseudotime units), and an early decline followed by an apparent late "recovery" (slopes -0.202 then $+0.304$; model improvement $F(2,40) = 5.71$, $p = 0.0066$; R^2 increase 0.074 to 0.280) [r1-46]. The later timing relative to the proteasome situates lysosomal acidification failure downstream in the cascade, while the late "recovery" may reflect sampling heterogeneity or selective survival of neurons retaining lysosomal integrity rather than true functional restoration [r1-46, r1-24, r1-80, r1-19].

Taken together, these data delineate a sequential failure of protein quality control arms in tau-positive neurons. We propose the following sequence: (1) As tau misfolds (rising MC1), neurons upregulate UPS components (proteasome subunits) but cross an early inflection at pseudotime ~0.37, after which proteasome capacity declines, promoting accumulation of ubiquitylated substrates and engaging selective autophagy (SQSTM1 up) [r1-24, r1-1]. (2) The autophagy–lysosome system initially compensates, with V-ATPase subunits upregulated in tau-positive versus tau-negative neurons; yet within tau-positive cells, increasing MC1 pushes the system past a

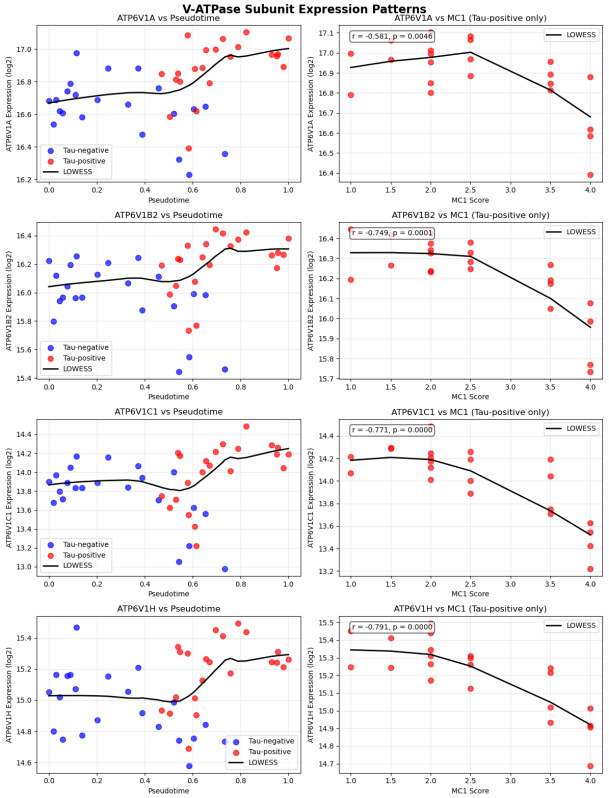

Figure 4: V-ATPase subunits exhibit a biphasic expression pattern indicative of a failed compensatory response to tau pathology. Expression of representative subunits increases along a disease pseudotime trajectory in tau-positive neurons (red points) relative to tau-negative neurons (blue points). Within the tau-positive population, however, subunit expression declines significantly with an increasing MC1 misfolded tau score following an initial upregulation. This biphasic trajectory is consistent with a model of initial compensatory upregulation of the lysosomal acidification machinery that collapses as the burden of misfolded tau progresses. (Source: [Trajectory r1-19])

collapse threshold at MC1 ≈ 2.831, consistent with a sudden loss of lysosomal acidification capacity and impaired autophagic flux [r1-19, r1-80]. (3) Along the disease trajectory, the V-ATPase breakpoint occurs later (pseudotime ~0.65) than the proteasome's, placing lysosomal failure downstream of proteasomal dysfunction; the apparent late "recovery" in pseudotime may reflect selective survival rather than restoration of function, further linking acidification competence to neuronal persistence [r1-46, r1-24]. This cascade coincides with a broad proteomic downshift—including reduced SLC2A1 (glucose transport), LMNA (nuclear architecture), and collagens—that plausibly amplifies metabolic stress, nuclear instability, and cell–matrix disengagement, thereby coupling tau accumulation to progressive dysfunction and dif-

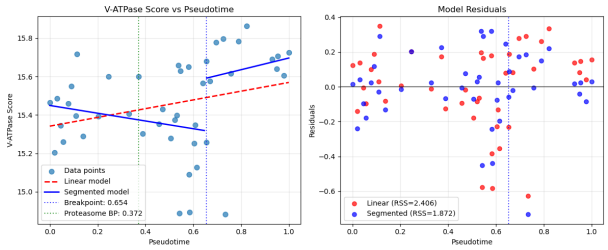

Figure 5: V-ATPase expression shows a late-stage biphasic response across disease pseudotime. (A) A segmented regression model (blue line) better describes the V-ATPase score versus pseudotime than a linear model (red line), identifying a breakpoint at 0.654 (blue dotted line). This breakpoint occurs subsequent to the proteasome breakpoint (green dotted line). (B) Residuals for the linear (red) and segmented (blue) models confirm the improved fit of the segmented model, as shown by its lower residual sum of squares (RSS). This late disruption of the V-ATPase system supports a sequential breakdown of proteostasis, with lysosomal failure following an earlier proteasomal dysfunction. (Source: [Trajectory r1-46])

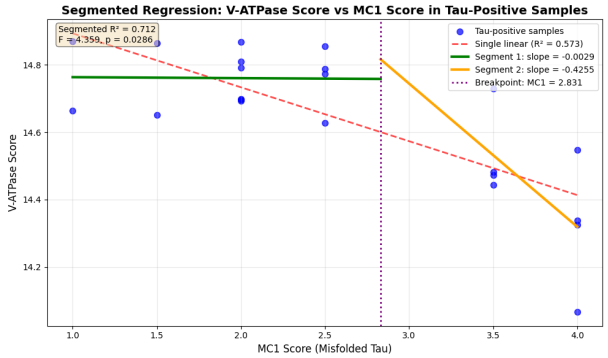

Figure 6: V-ATPase expression collapses beyond a critical threshold of misfolded tau pathology. Segmented regression analysis of the V-ATPase score versus the MC1 (misfolded tau) score reveals a stable expression phase followed by a steep decline after a breakpoint at an MC1 score of 2.831. This biphasic response is consistent with a failed-compensation model where the lysosomal acidification machinery fails at advanced stages of tauopathy. (Source: [Trajectory r1-80])

ferential survival outcomes [r1-1]. Mechanistically, these results support a model wherein early proteasomal insufficiency seeds proteotoxic load and autophagic dependence, followed by a thresholded failure of lysosomal acidification that curtails flux, locks in tau accumulation, and accelerates cellular decline [r1-24, r1-80, r1-19, r1-46, r1-1].

Trajectory Sources

Trajectory r1-1: A comprehensive differential protein expression analysis identified 2,115 proteins (36.14% of total) significantly differentially expressed between tau-positive and tau-negative neurons, with 1,288 down-regulated and 827 up-regulated proteins in tau-positive samples.

Trajectory r1-19: All four V-ATPase complex subunits (ATP6V1A, ATP6V1B2, ATP6V1C1, and ATP6V1H) exhibit the predicted biphasic expression pattern, being significantly upregulated in tau-positive samples but showing strong negative correlations with MC1 score, confirming the hypothesis of compensatory upregulation fol...

Trajectory r1-24: The expression of proteasome core subunit proteins shows a statistically significant non-linear pattern along the pseudotime axis, with a segmented regression model identifying a critical breakpoint at pseudotime = 0.3724 (p = 0.0031) where proteasome expression transitions from increasing (+1.33 sl...

Trajectory r1-46: The V-ATPase system exhibits a significant biphasic relationship with pseudotime (F-test p-value = 0.0066), but the breakpoint occurs at pseudotime 0.654, substantially later than the proteasome breakpoint of 0.372.

Trajectory r1-80: The V-ATPase Score exhibits a significant biphasic pattern in tau-positive samples, with a compensate-then-collapse response that transitions at MC1 score 2.831.