The Link Between Mitochondrial Dysfunction and Neurodegeneration

Roles of Parkin, PINK1, and Mitochondria in Parkinson's Disease

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Neurodegeneration

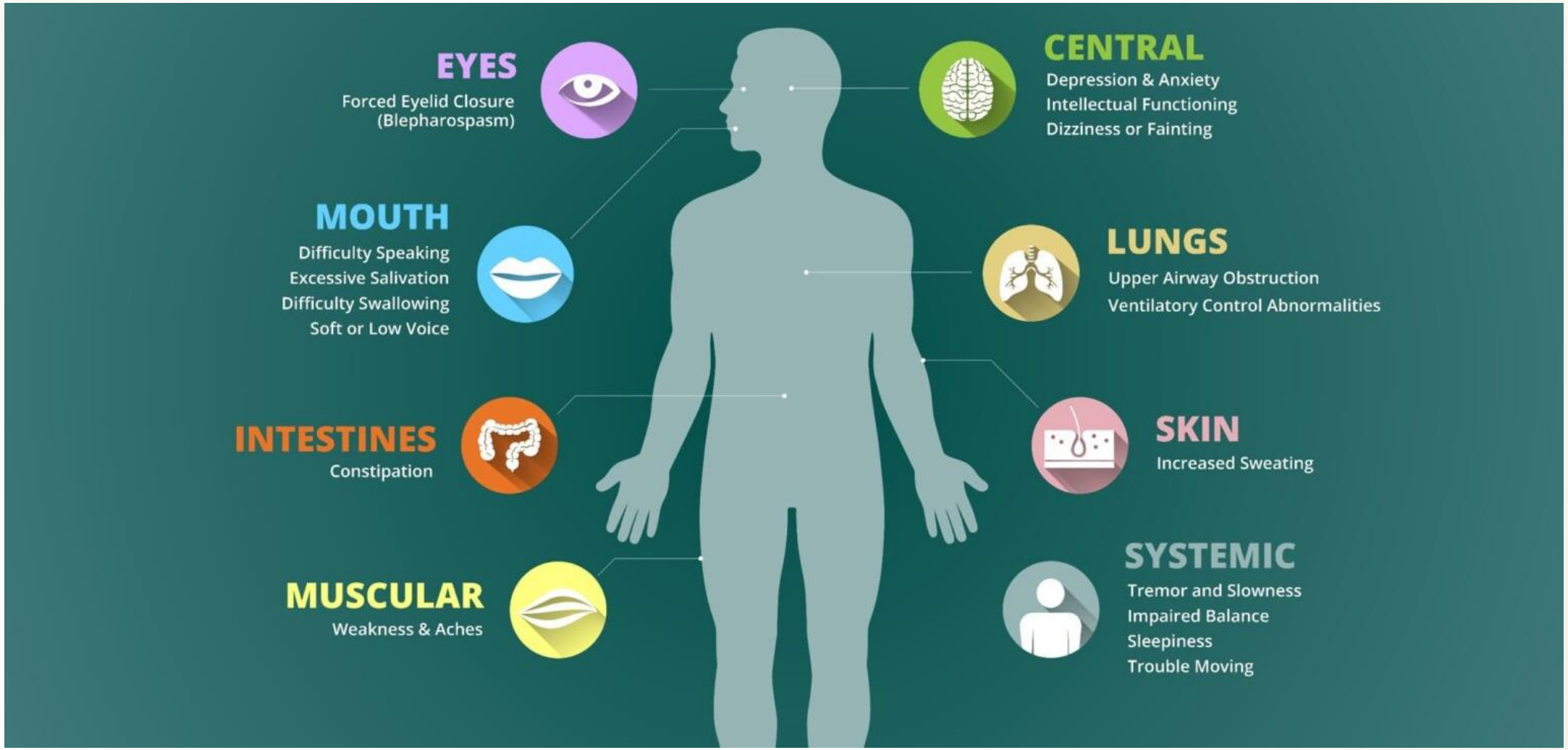
- Neurodegeneration is defined as slow and progressive loss of neuronal cells, culminating in cell death.

- Parkinson's Disease (PD) and Alzheimer's Disease (AD):
  - Characterized by accumulation of aggregated proteins and defective mitochondria, leading to neuronal cell death.

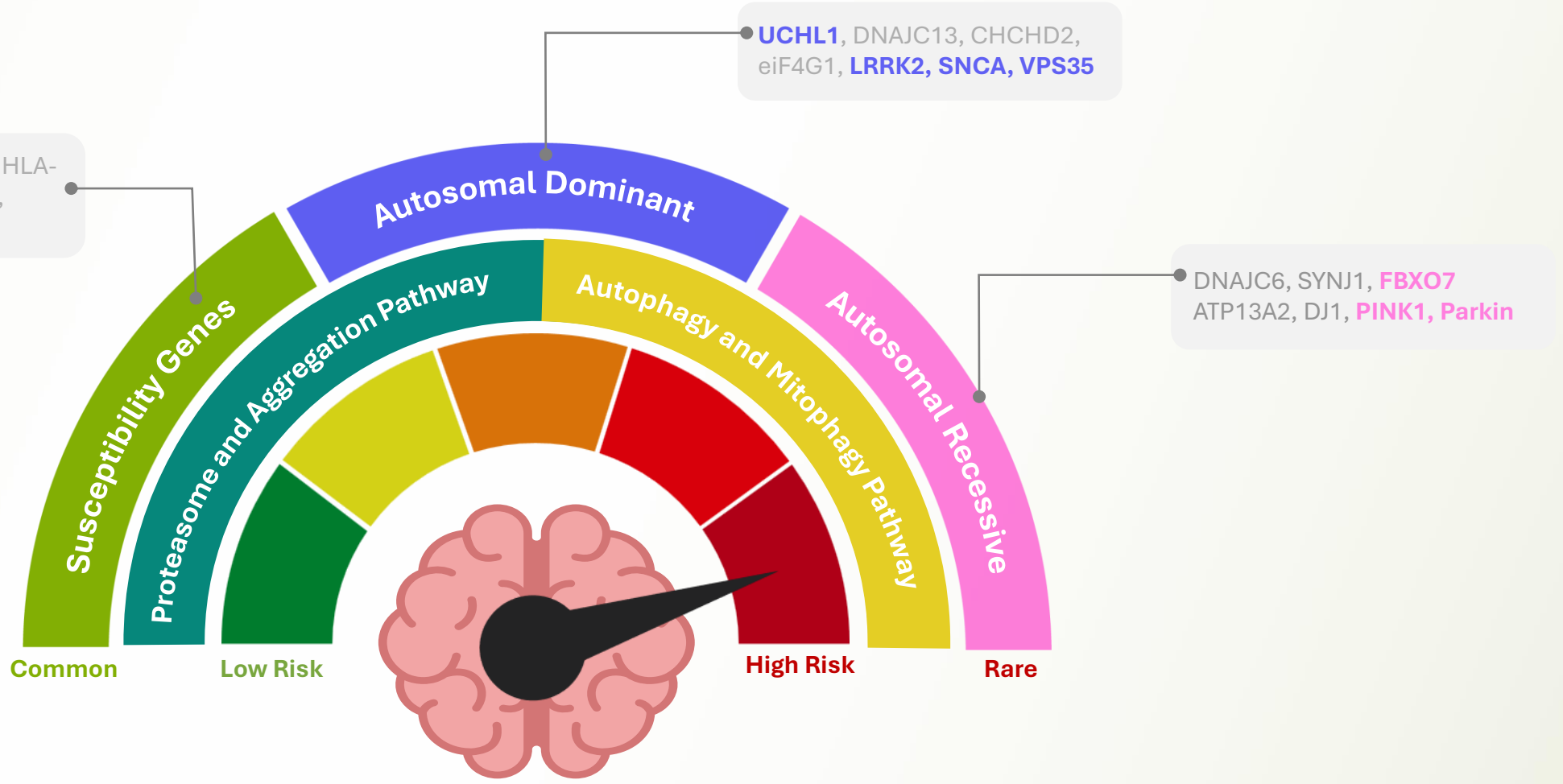
- The Role of Cellular Pathways:
  - Ubiquitin Proteasome System (UPS) and Autophagy-Lysosomal Pathway (ALP) are crucial in neurodegenerative diseases.

- Therapeutic Potential: Targeted degradation of aggregated proteins and damaged mitochondria holds promise for neuroprotection and transformative therapies in PD and AD.

Parkinson's Disease: More than a Movement Disorder



Lessons from Genetics - PD



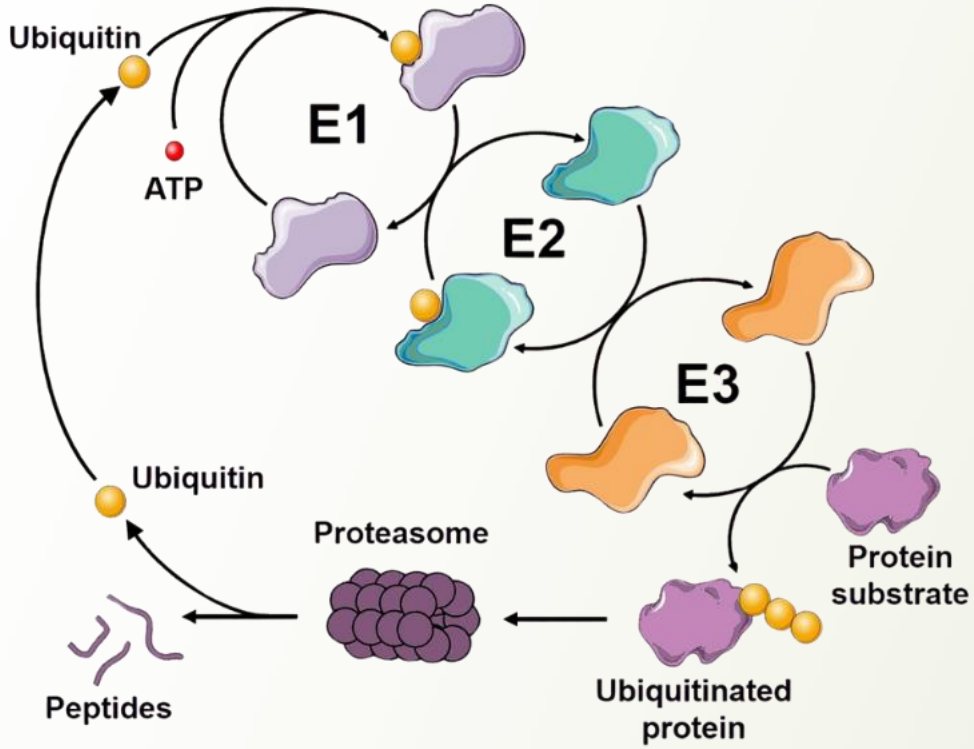
E3 Ligase Dependency - Neurodegeneration

E3 ligases and their roles in neurodevelopmental diseases

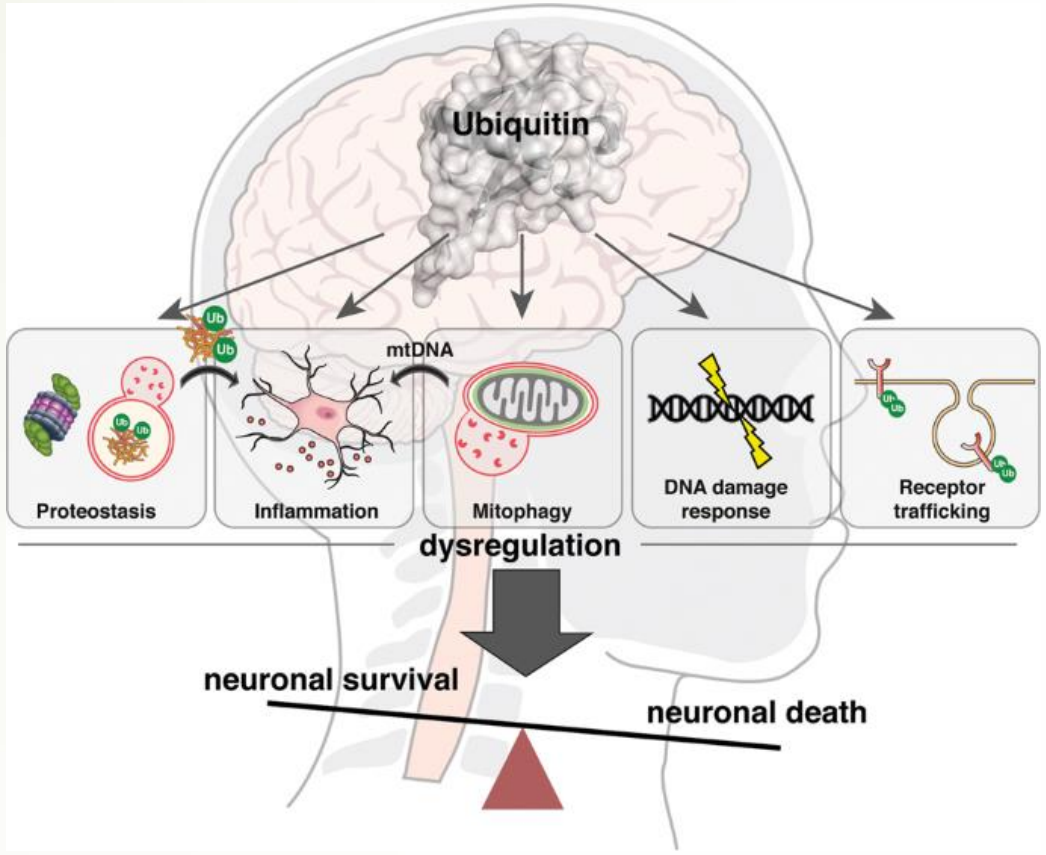
E3 ubiquitin ligases in neurological diseases: focus on gigaxonin and autophagy. Frontiers in physiology, 11, 568645.
Ubiquitin signalling in neurodegeneration: mechanisms and therapeutic opportunities. *Cell Death & Differentiation*, *28*(2), 570-590.

Ubiquitin Proteasome System – The Guardian of the Cell

- Dysfunction in the Ubiquitin Proteasome System (UPS) leads to aggregation of mis-folded proteins, such as tau and α-synuclein.
- Protein aggregates disrupt mitochondrial membranes, causing mitochondrial depolarization.
- Dysfunctional mitochondria and loss of ATP is the main cause of neuronal cell death.



Ubiquitin Proteasome System (UPS) and Parkinson's Disease



Degradation & Non-degradative Ubiquitin Signaling

Progressive loss of neurons vs UPS

- One of the most overlooked and major component of proteinopathies is **ubiquitin**.
- Ubiquitin here is either an adaptive response to toxic misfolded proteins or evidence of dysregulated UPS driving aggregation.

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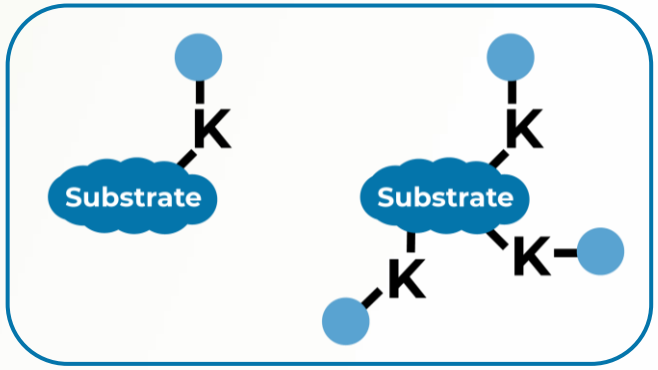
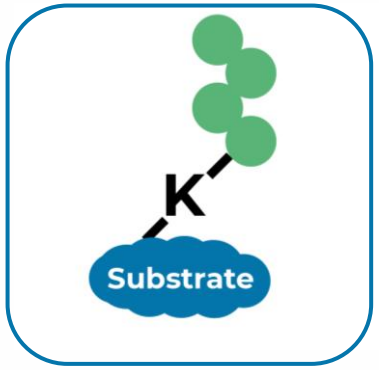
Ubiquitin Code

Mono & Multi Mono-ubiquitination



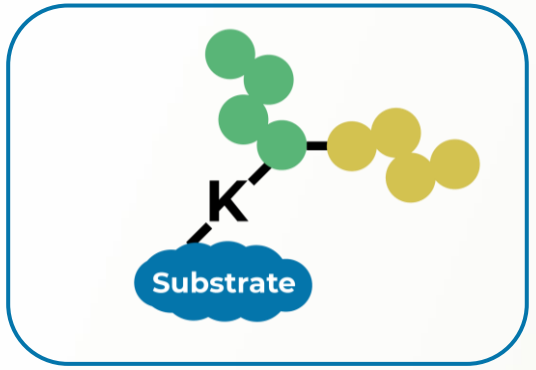
Protein Sorting &
Histone modifications

Homotypic Chains



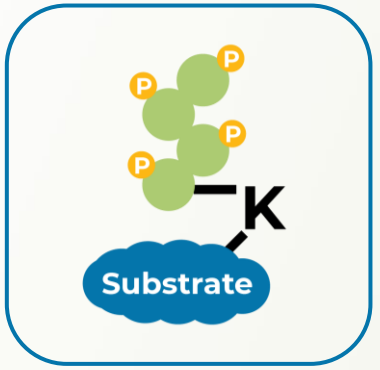
(M1, K6, K11, K27, K29, K33, K48, K63)
&
Degradation & Signalling

Heterotypic Chains



Enhanced Degradation & Signalling

Phospho-Ubiquitin

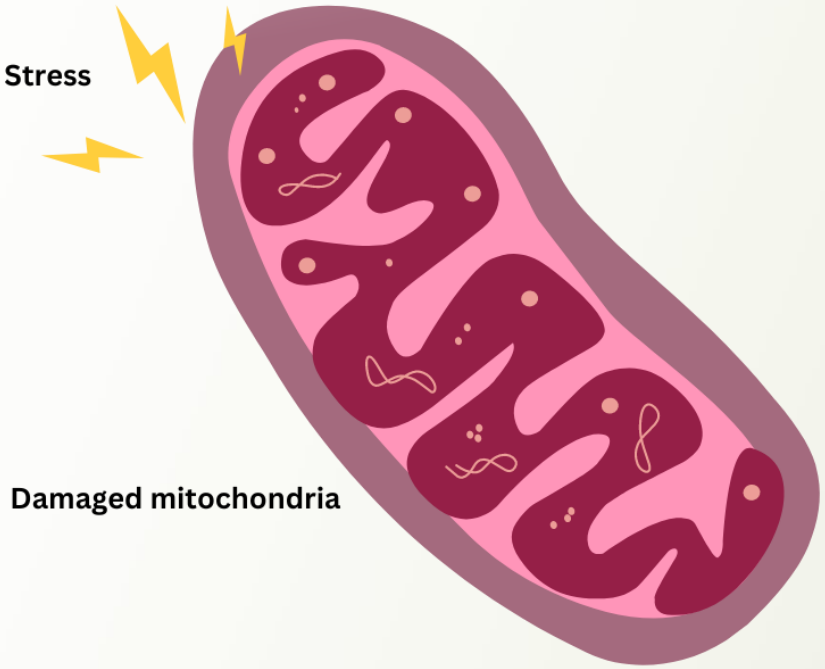


Signalling
&
Mitophagy

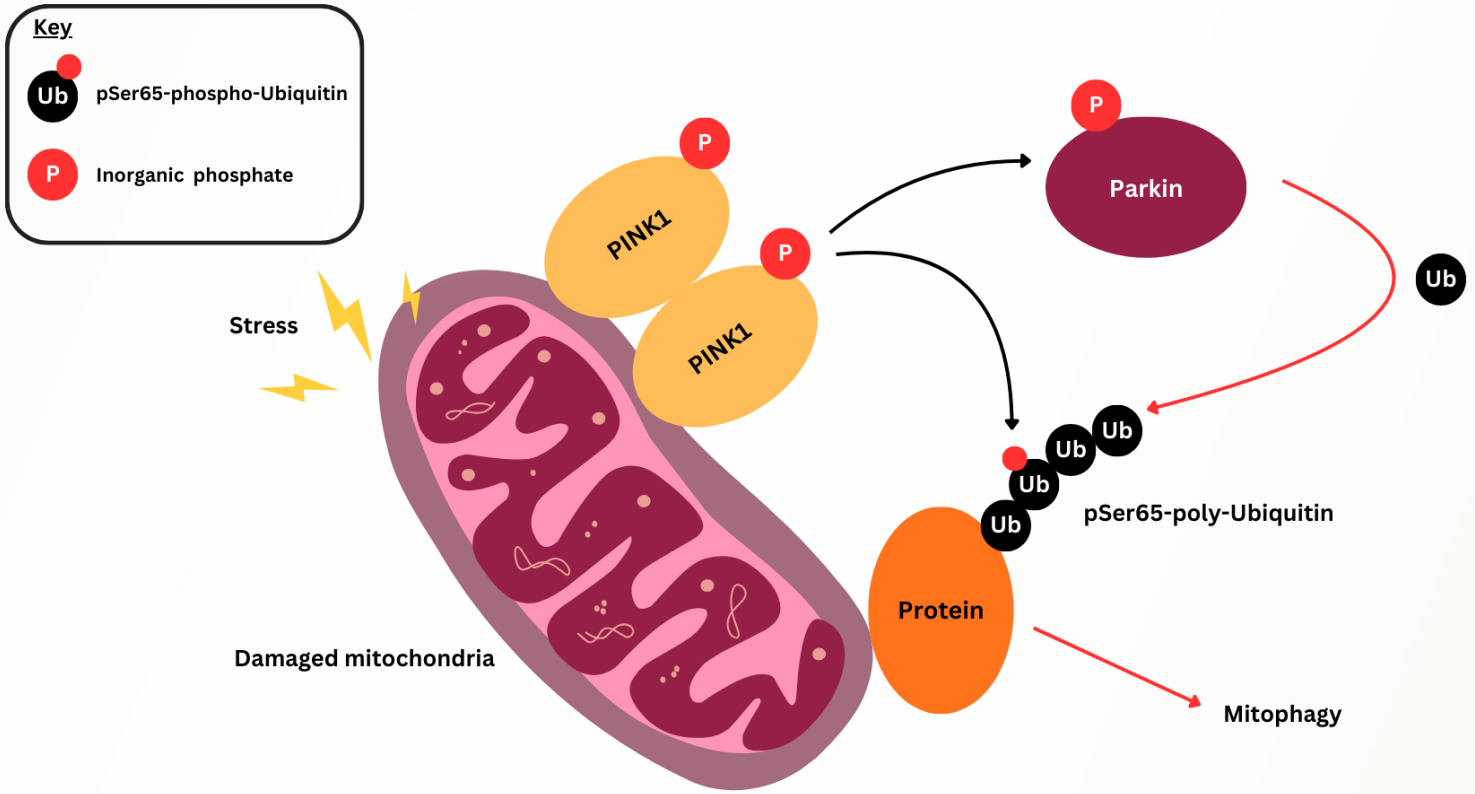
Degradation, **Interactions**, **Localization** & **Signaling**

Mitochondrial Health is Crucial in Neurodegeneration and PD

- Mitochondrial health is pivotal in neurodegeneration and PD, impacting energy supply and oxidative stress.
- PINK1 and parkin ligase regulates mitochondrial dynamics quality control, initiating mitophagy to remove damaged mitochondria.
- Mutations in PINK1 and parkin genes are linked to familial Parkinson's disease.
- Phospho-ubiquitin aids in tagging damaged mitochondria for degradation during mitophagy, crucial for preventing neurodegeneration.



Phospho-ubiquitin: A Hallmark of Neurodegeneration

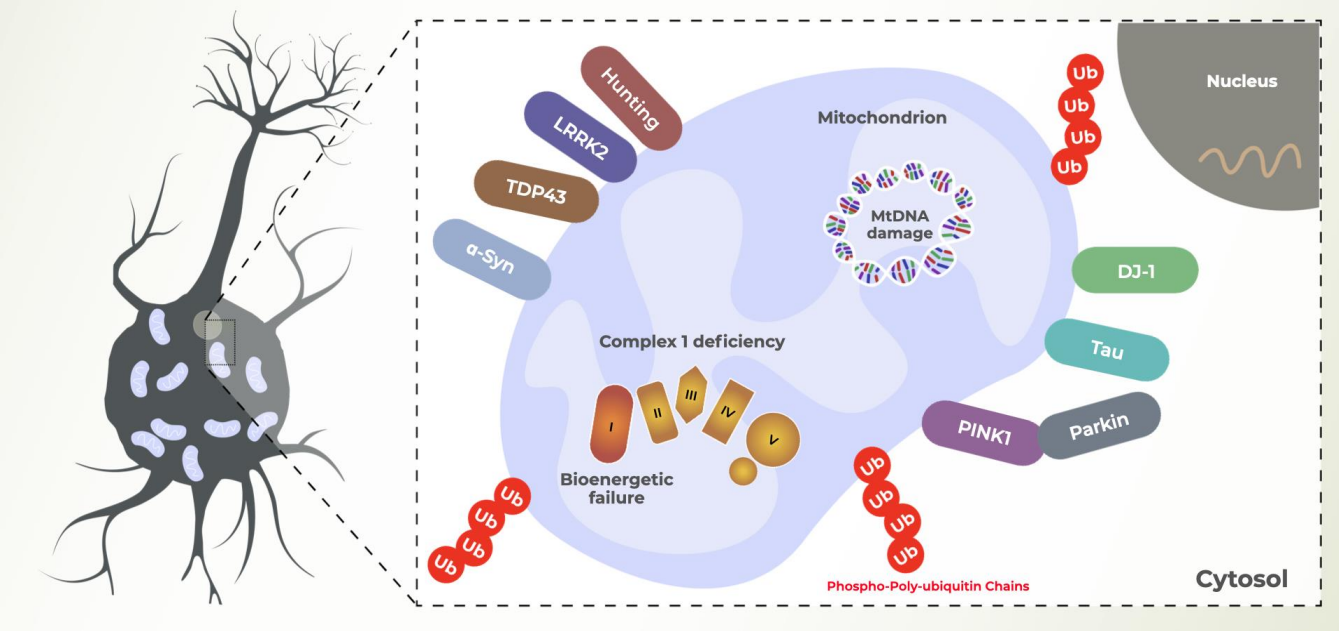


- Within the mitochondrial membrane, the **PINK1** kinase becomes activated, triggering the phosphorylation of ubiquitin and parkin ligase.

- **Parkin ligase** then attaches phospho-ubiquitin (pUb) to the outer mitochondrial membranes, initiating mitophagy, the process of removing damaged mitochondria and promoting new mitochondria generation (mitobiogenesis).

Protein Aggregation and Mitochondrial Damage

- Proteinopathies cause damage to mitochondria
- Many neurodegenerative diseases have protein aggregation problems in neurons
- Increase in phospho-ubiquitin is an indicator of neurodegeneration
- Development of blood-based phospho-ubiquitin key goal



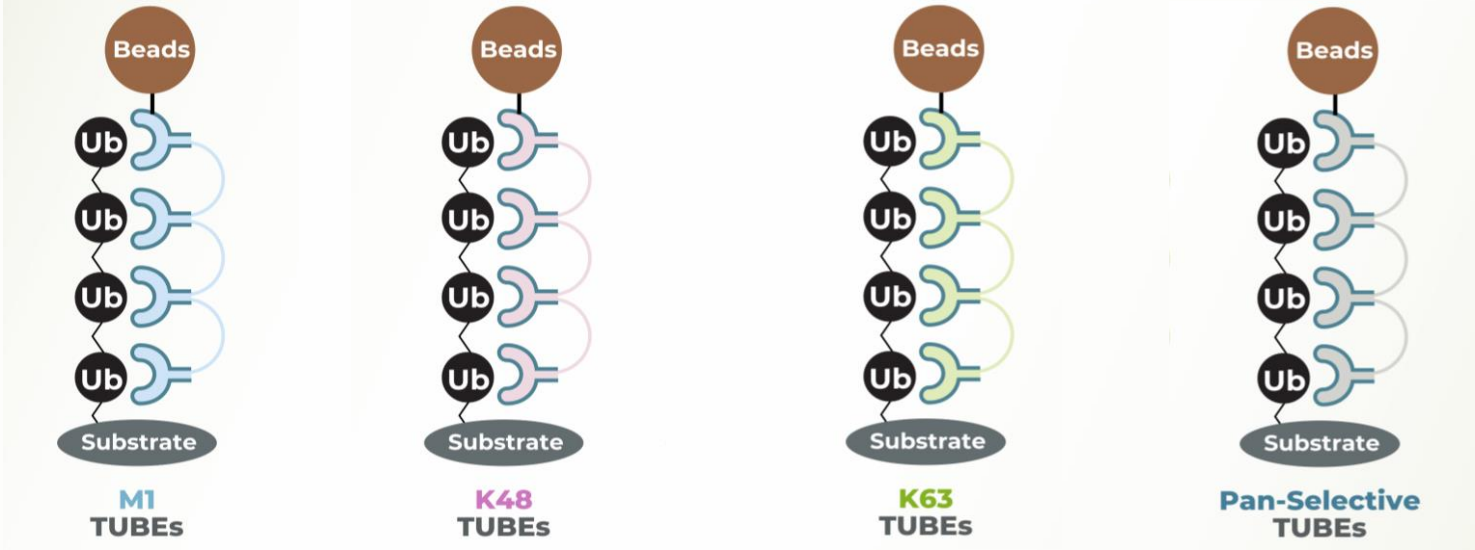
Are Tools Available for UPS Biomarker Discovery?

Ubiquitin antibodies are not suitable for enrichment studies in complex matrices !!!

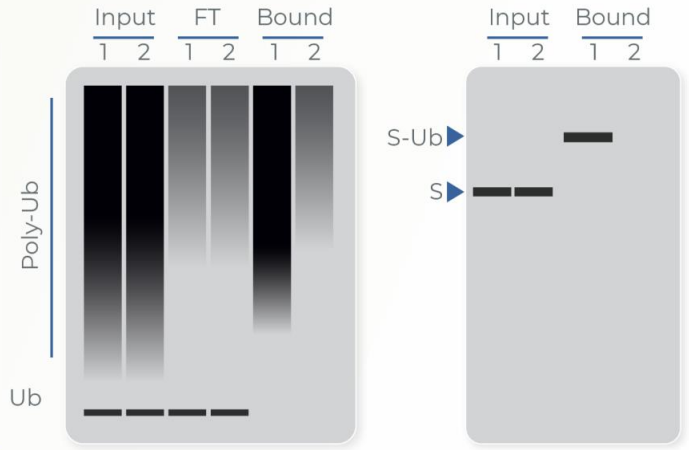
- Ubiquitin is highly conserved across species and is a poor antigen
- Phospho-ubiquitin antibody is monovalent
- Difficult to develop diagnostic applications
- Phospho-polyubiquitin chains are heterogenous and antibodies do not detect all epitopes
- Antibodies challenged by chain selectivity, especially in complex media
- Most importantly, antibodies do not immuno-precipitate phospho-polyubiquitinated proteins

TUBE Technology

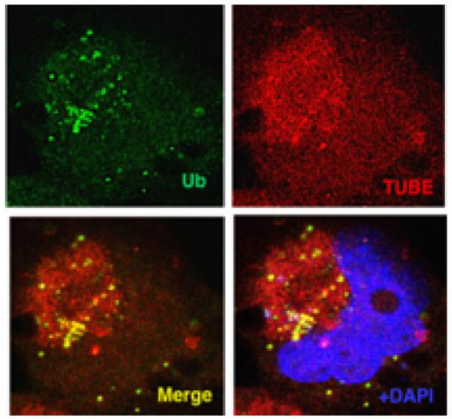
TUBE (Tandem Ubiquitin Binding Entities)



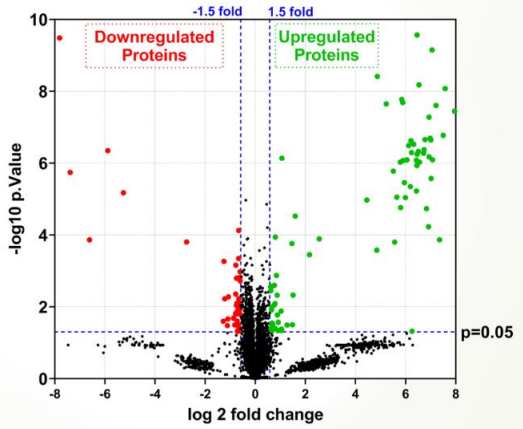
Poly-Ubiquitin Substrate Enrichment & Western Blotting



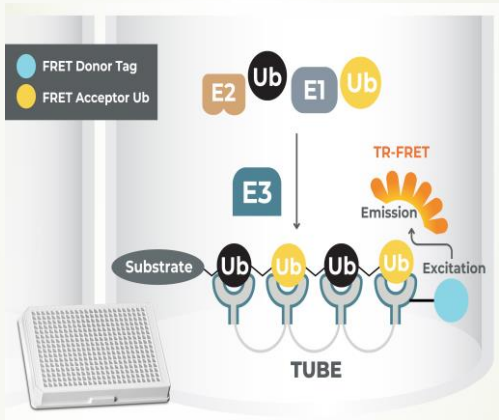
Fluorescent Microscopy



Mass Spectrometry Ubiquitomics



High-Throughput Screening

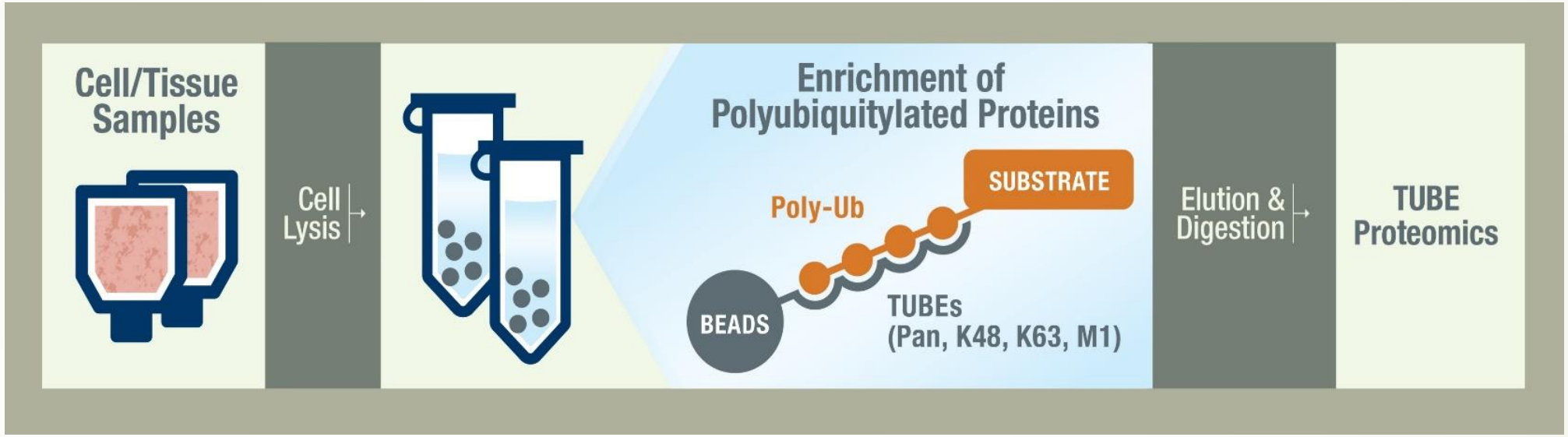


Phospho-TUBEs: Coming Soon…

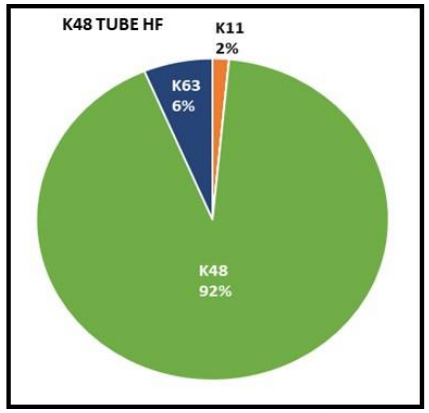
LifeSensors is developing novel phospho-ubiquitin TUBEs and other phospho-ubiquitin tools to unveil Parkinson's disease signature and discover novel PD biomarkers

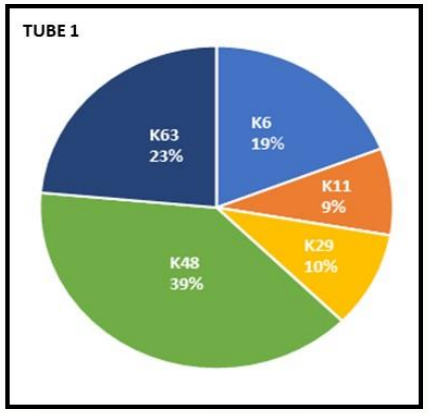
TUBE Mass Spec Proteomics

Pan and Chain Selective TUBEs with sub -nM Affinities



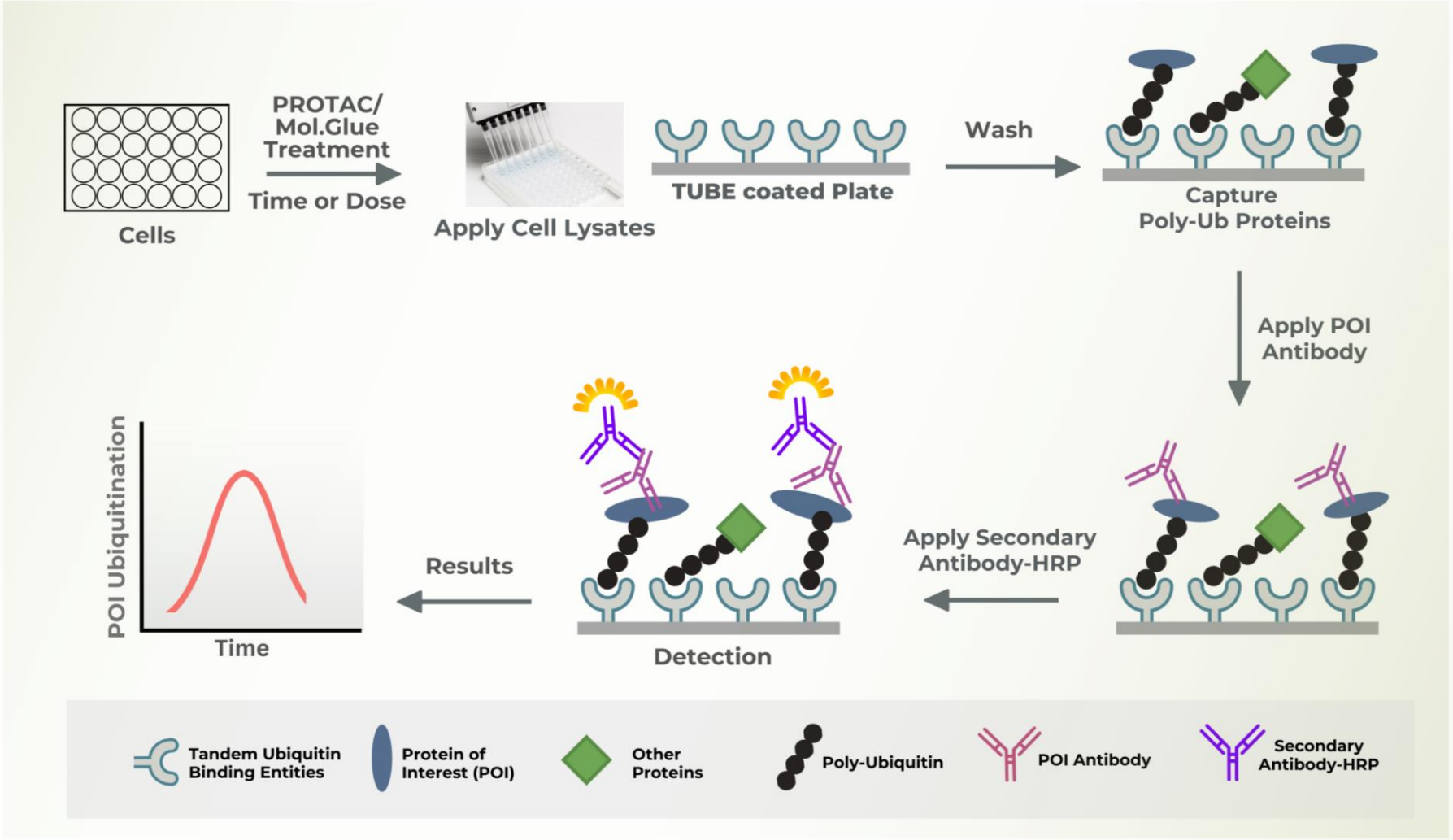
Remarkable selectivity of K48 TUBE HF for K48-linked ubiquitylated substrates in neuronal cells.





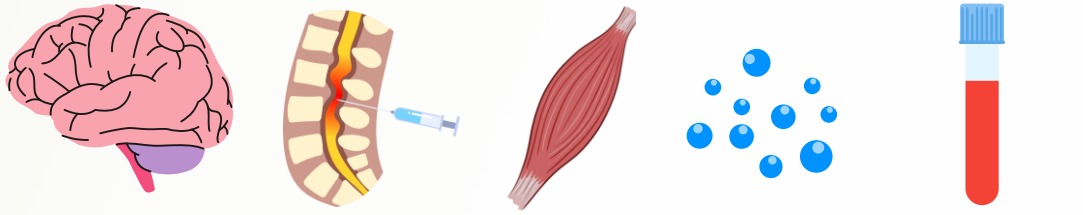
UbiQuant™ ELISA

Capturing ubiquitinated proteins on a microtiter plate for absolute quantitation



Our Vision

Universal Tools for PD Biomarker Discovery & Diagnostics



Proteomics

Biomarker Panel - Discovery

Diagnostic Panel

Our Commitment

A blood-based test to empower Patients, Practitioners & Researchers for better diagnostic solutions and PD Management

- ✓ Identifying elevated novel UPS biomarkers in Serum.
- ✓ Testing Phospho-S65-Ubiquitin levels in Serum for Familial PD.
- ✓ Harnessing activity data from genetic markers PINK1/Parkin in PBMCs to provide insights into Familial PD.

LifeSensors' Neurodegeneration Tools

- LifeSensors offers a suite of tools to support neurodegeneration research and help develop ultra sensitive assays for biomarker development and diagnostic applications:
  - Phospho-ubiquitin antibodies
  - Phospho-ubiquitin chains and derivatives (K48, K63, K11, K6, K33, M1)
  - Neuronal DUBs and Ligases
  - PINK1 & Parkin pathway proteins
  - Phospho-polyubiquitinated protein enriched lysates
  - TUBEs (Tandem Ubiquitin Binding Entities)
  - UbiQuant for monitoring phospho-polyubiquitination

Thank You

We are your partner in Parkinson's Disease and Neurodegeneration Research

Contact Us!

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