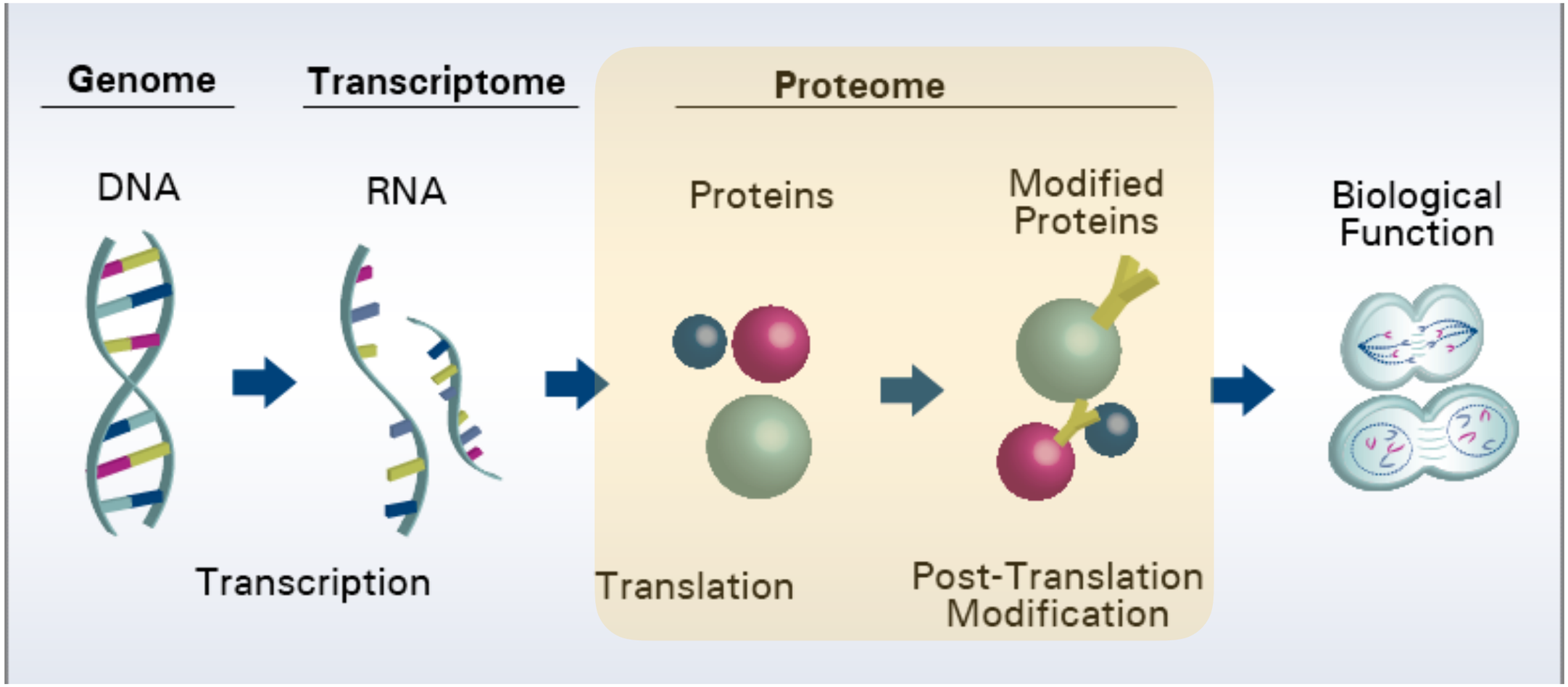
PROTEOMICS 2: QUANTITATIVE

Yukun Li, Kye Nichols, March 31st, Gen 564Sp20

REVIEW: What is proteomics?

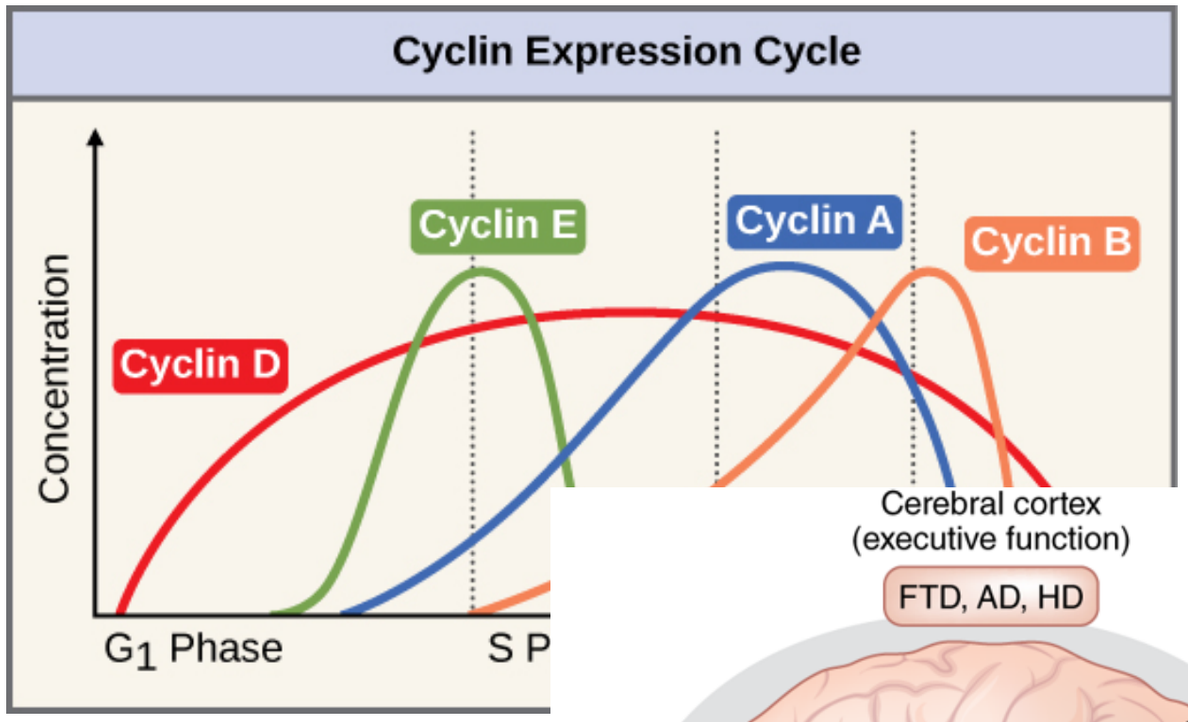


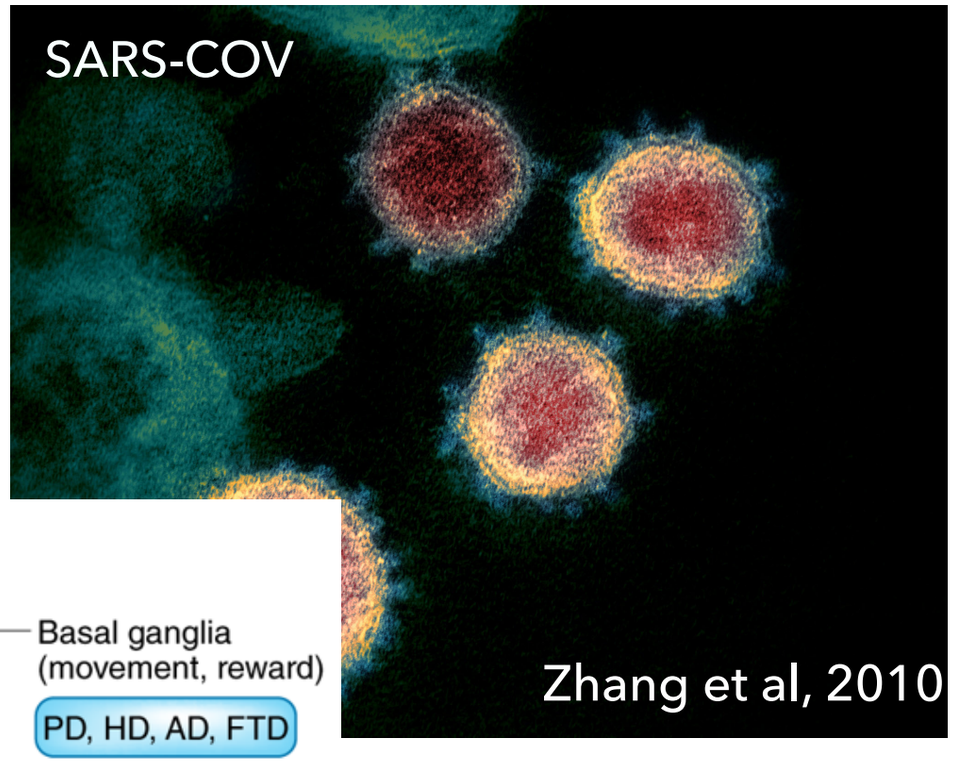
Proteomics : identify and quantify the proteome

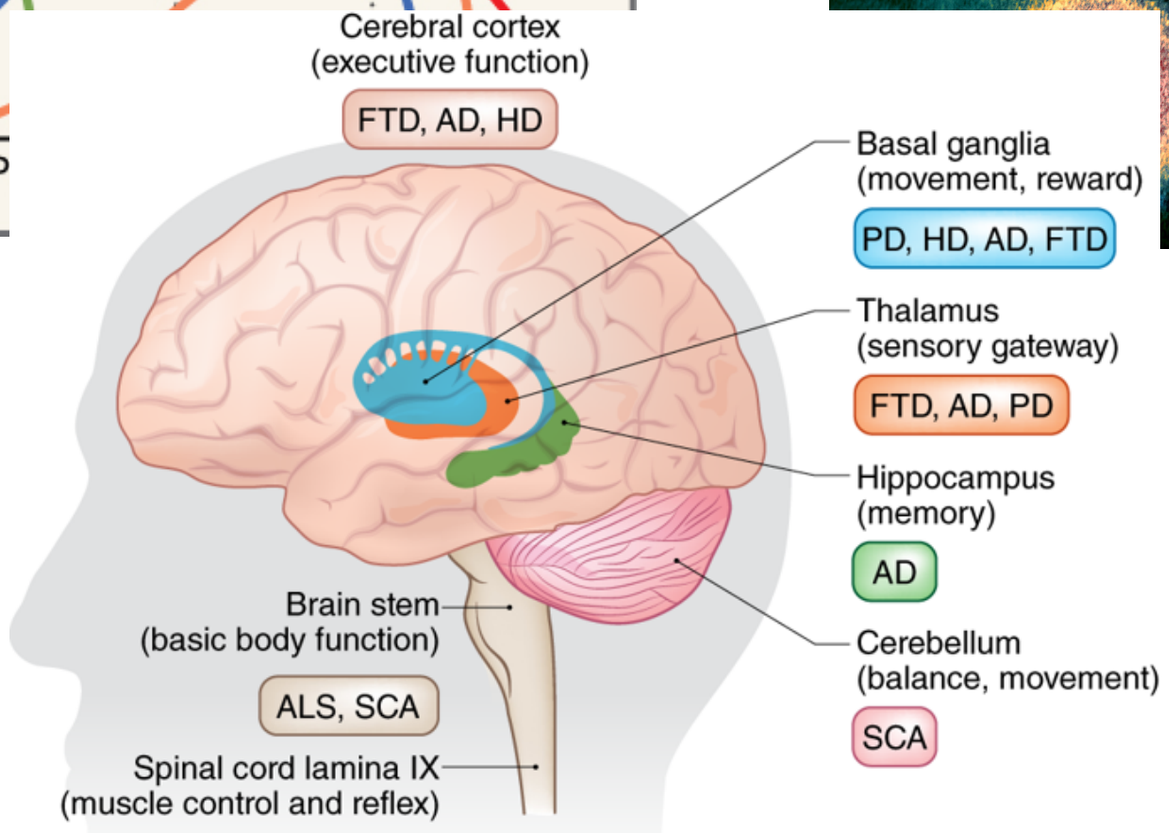
QUIZ:

Why are protein levels important?

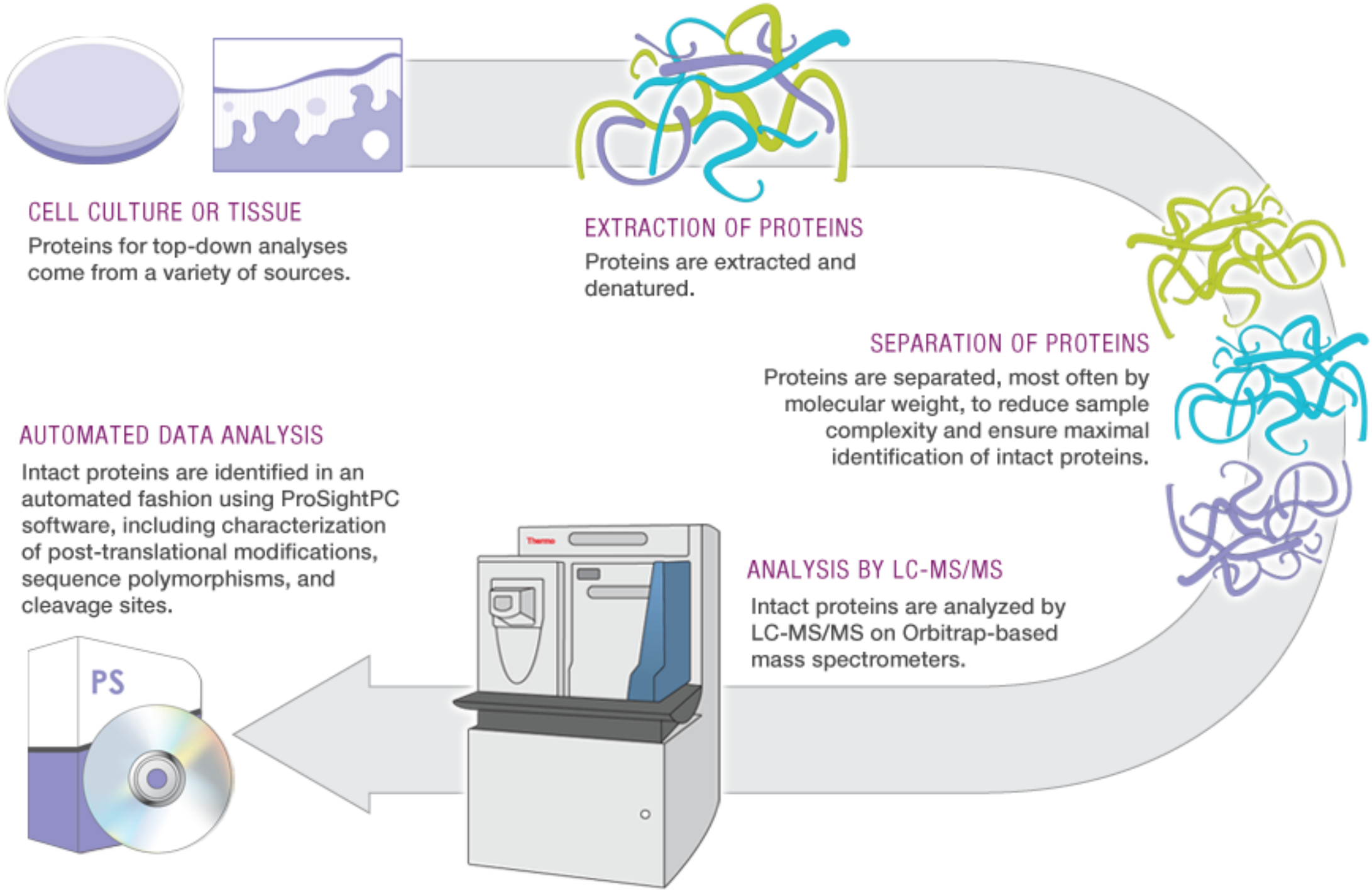
What biological processes are protein levels important?





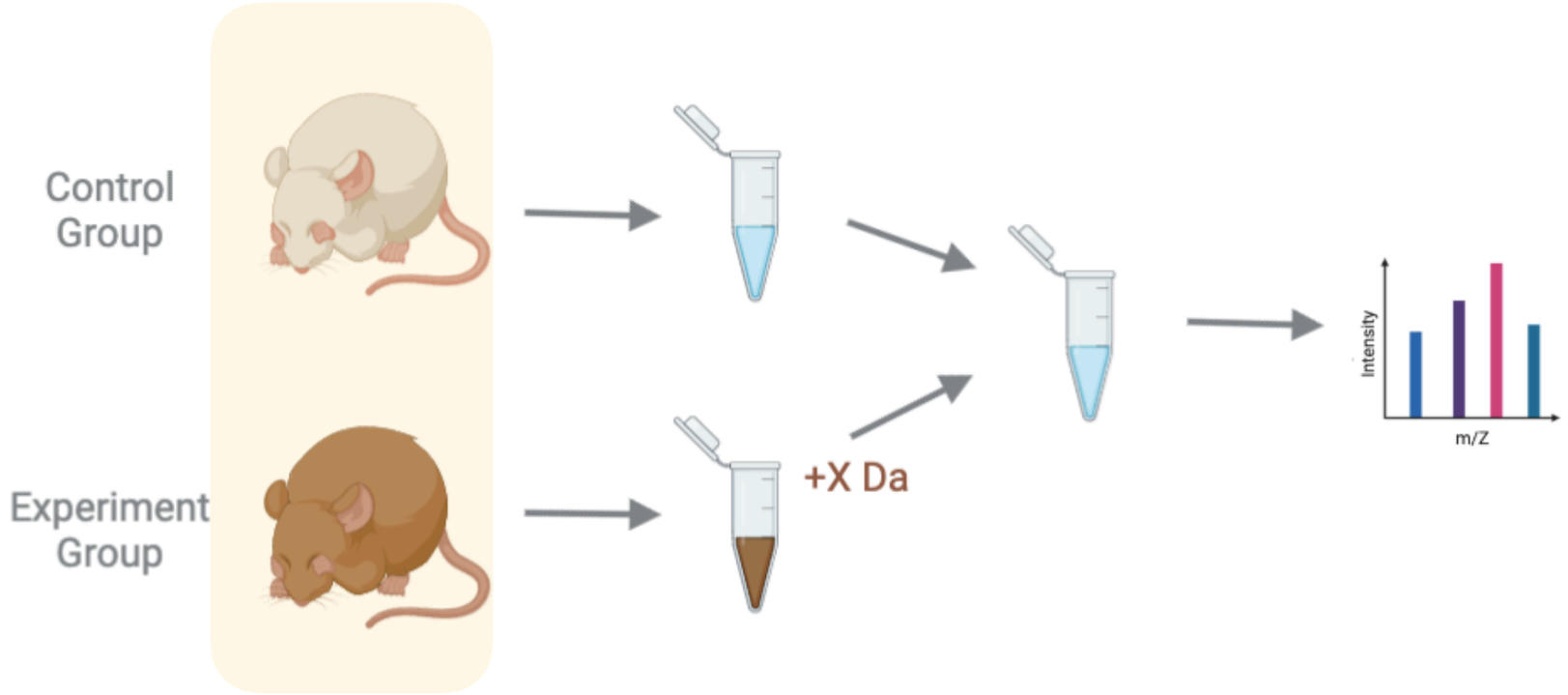


Another review: What is the workflow of proteomics studies?



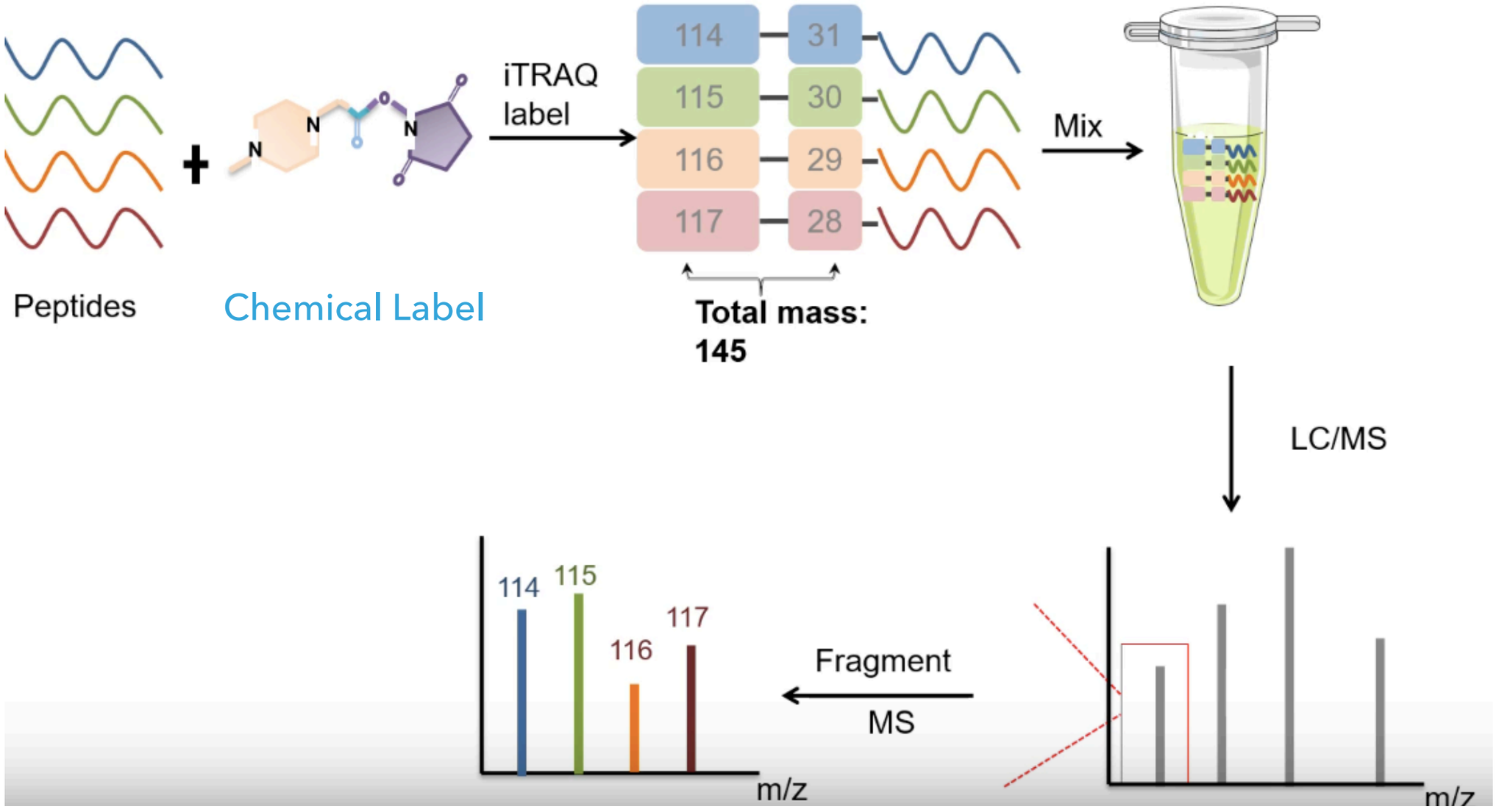
What are methods to study quantitative proteomics?

Chemical Label



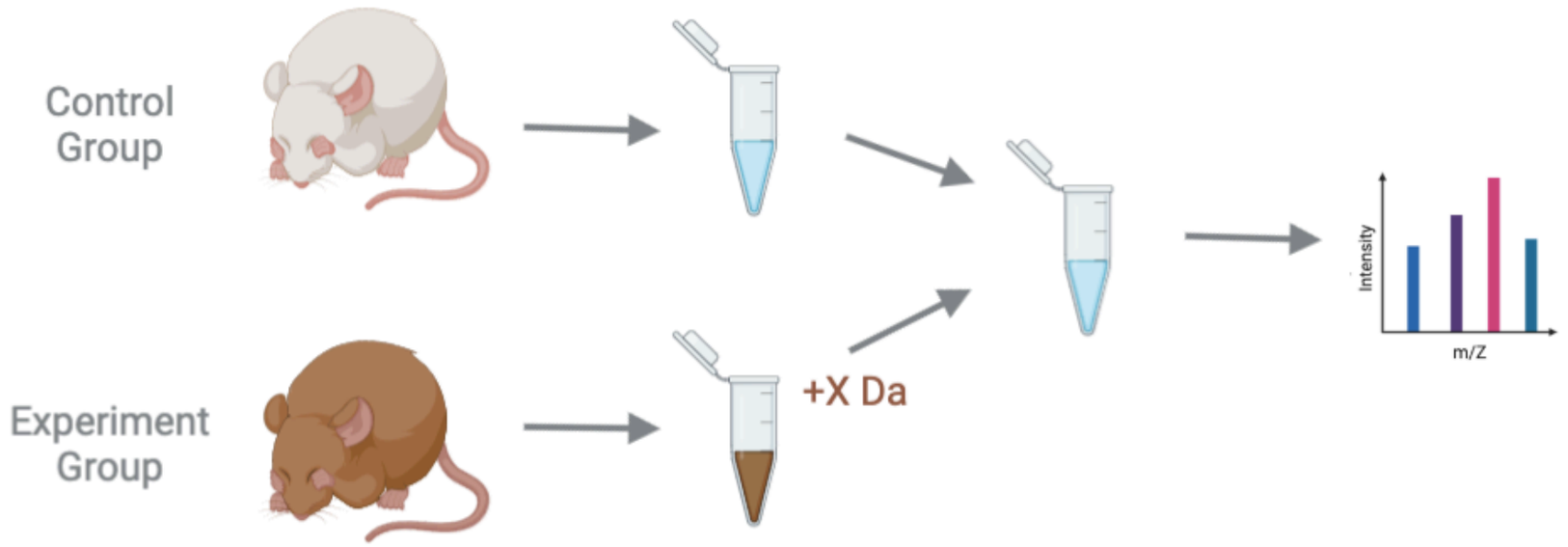
What is iTRAQ ?

Isobaric tag for relative and absolute quantitation

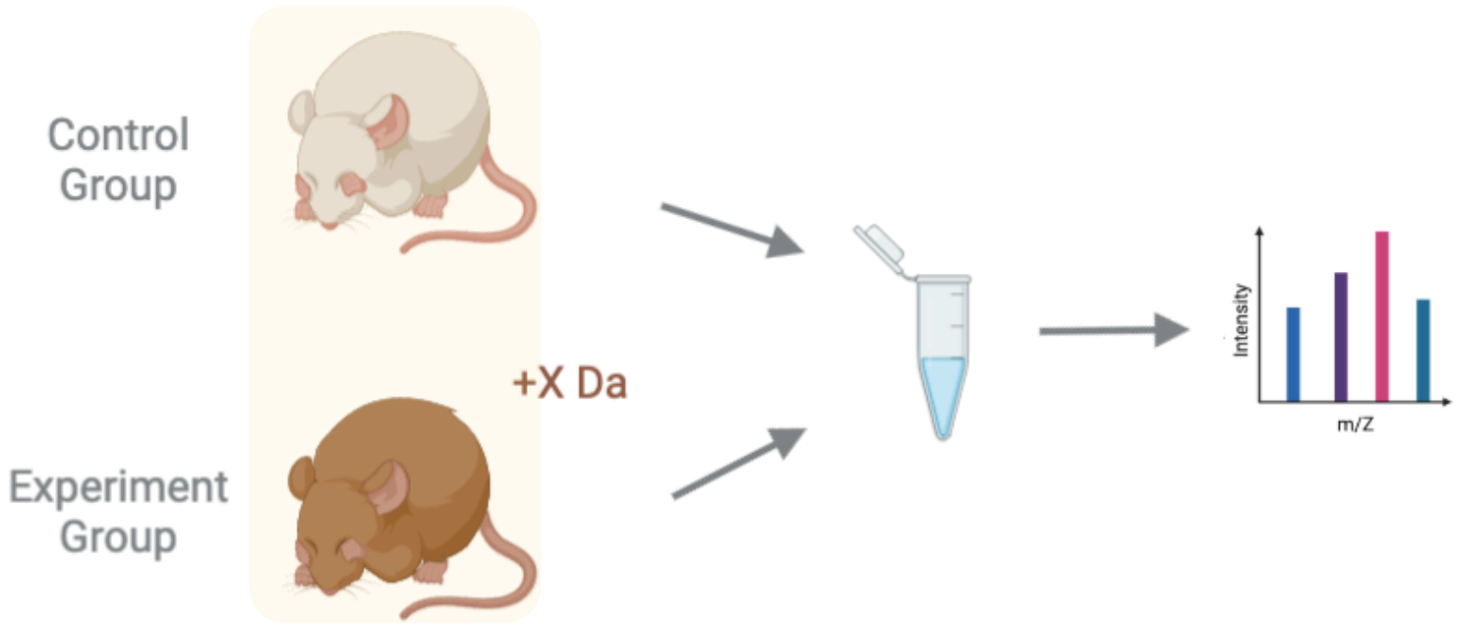


What are Quantitative ways to study proteomics ?

Chemical Label



Metabolic Label



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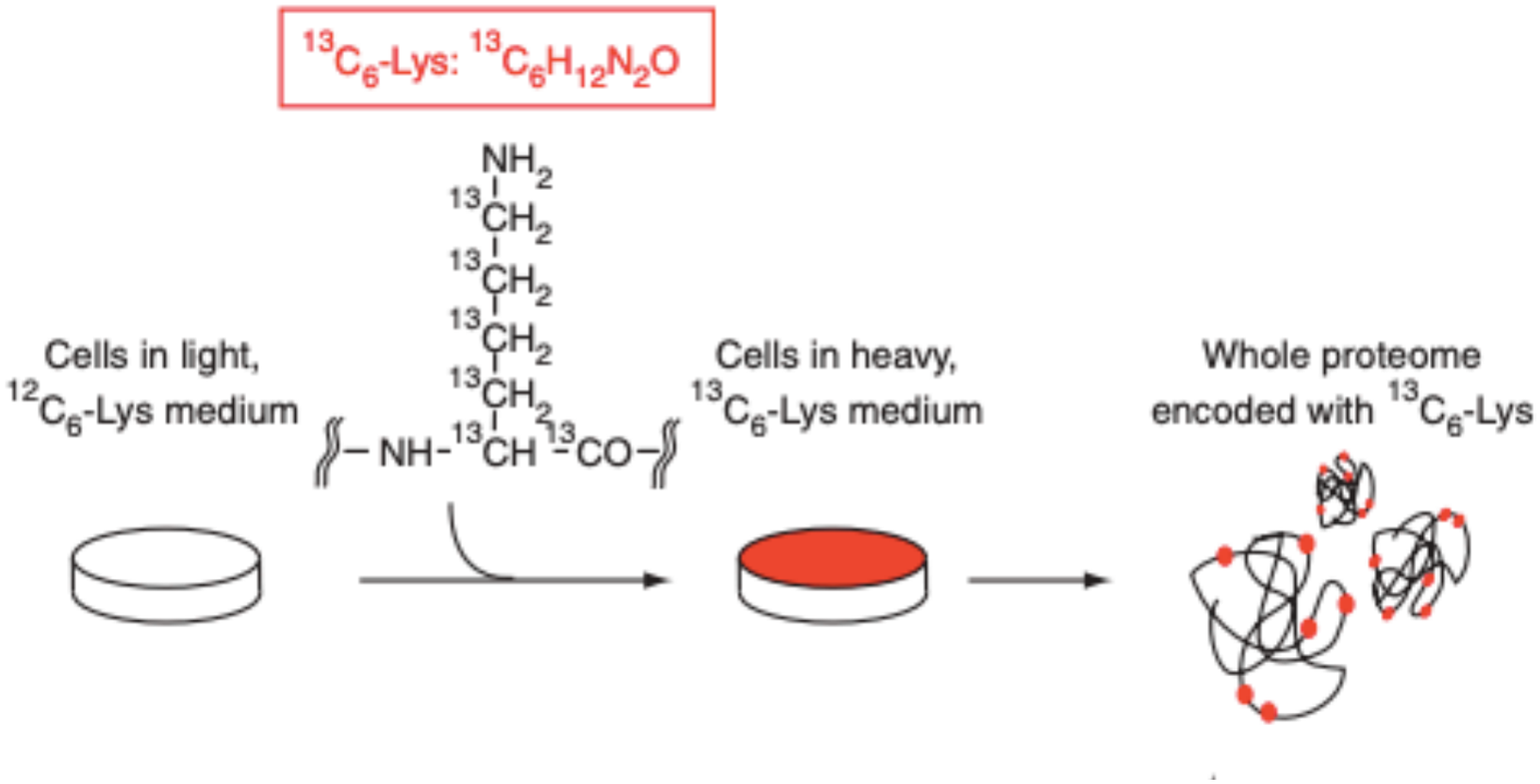
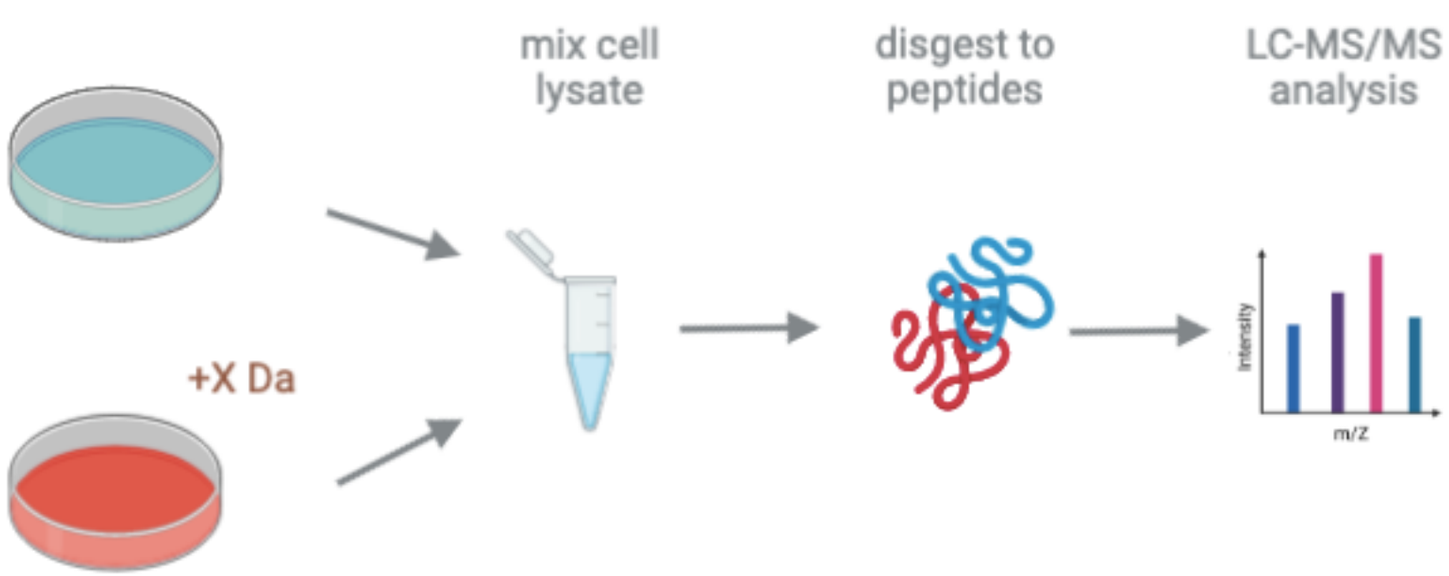
What is the general overview of SILAC?

Adaption Phase



Experiment Phase



What is the Adaption Phase of SILAC?

Medium Preparation

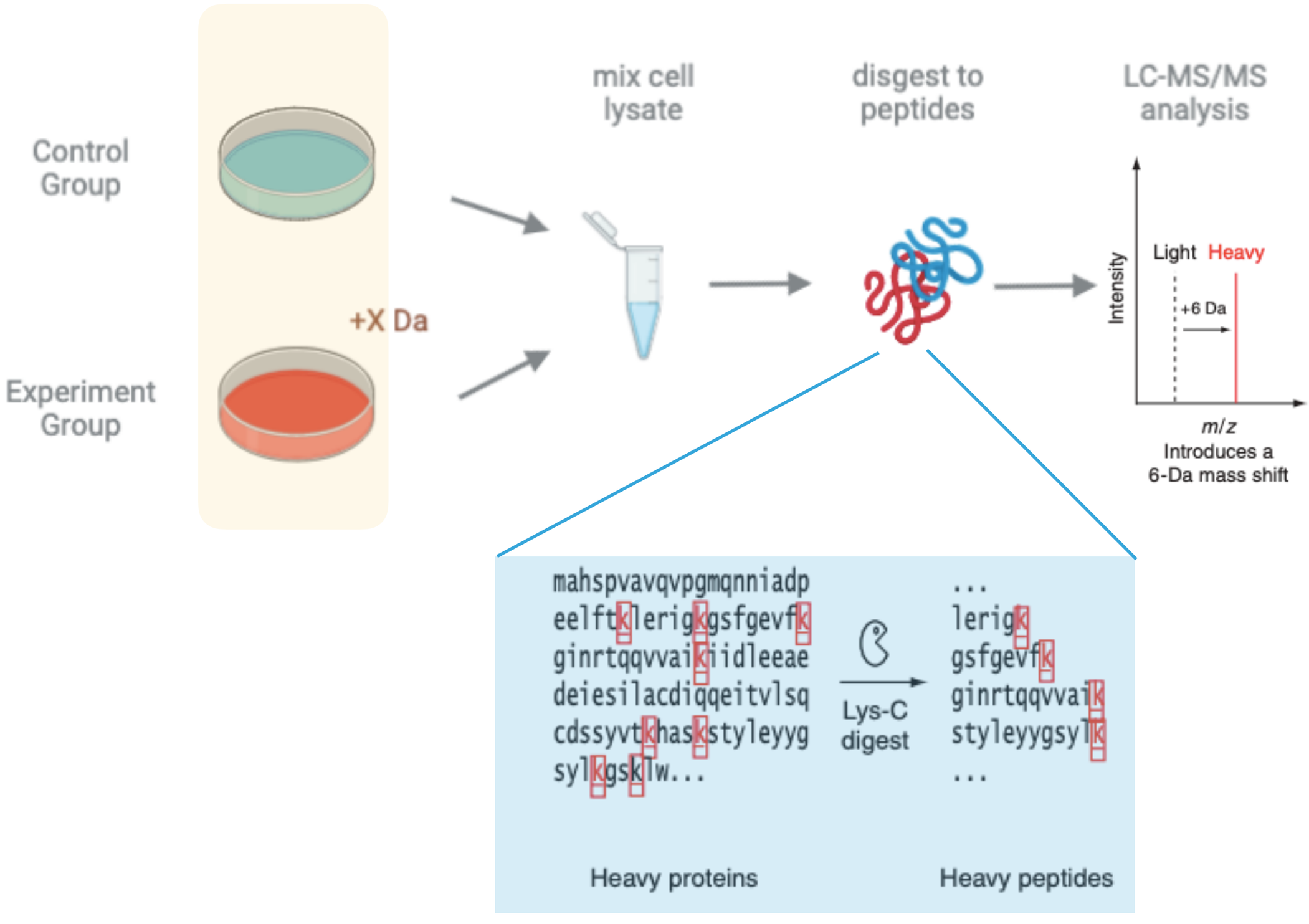
light

heavy

- amino acid essential for cell survival
- arginine and lysine often used
- C13, N15, H2

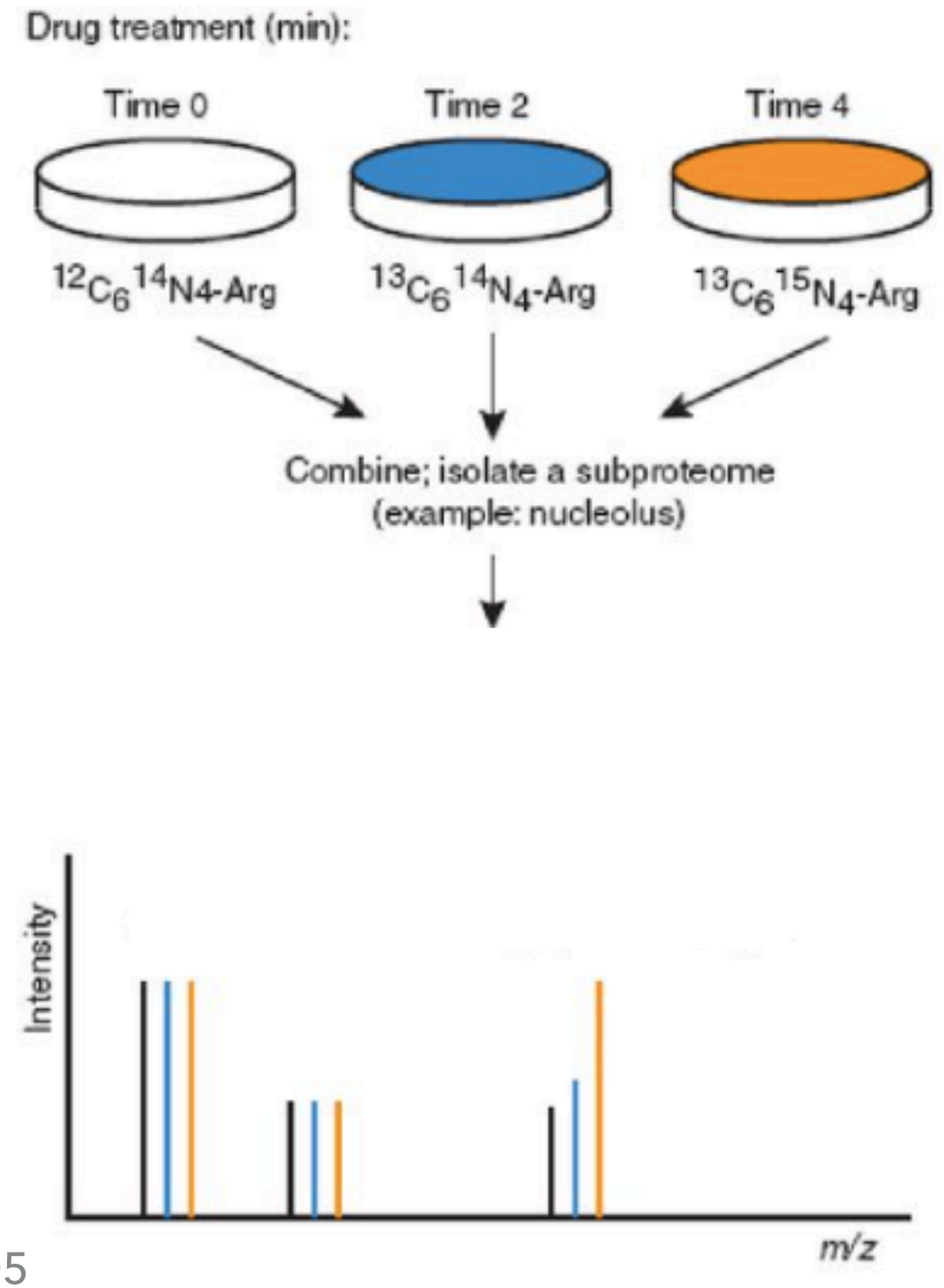
Ong et al., Mol Cell Proteomics , 2002

What is the experiment phase of SILAC?



What if you had multiple samples?

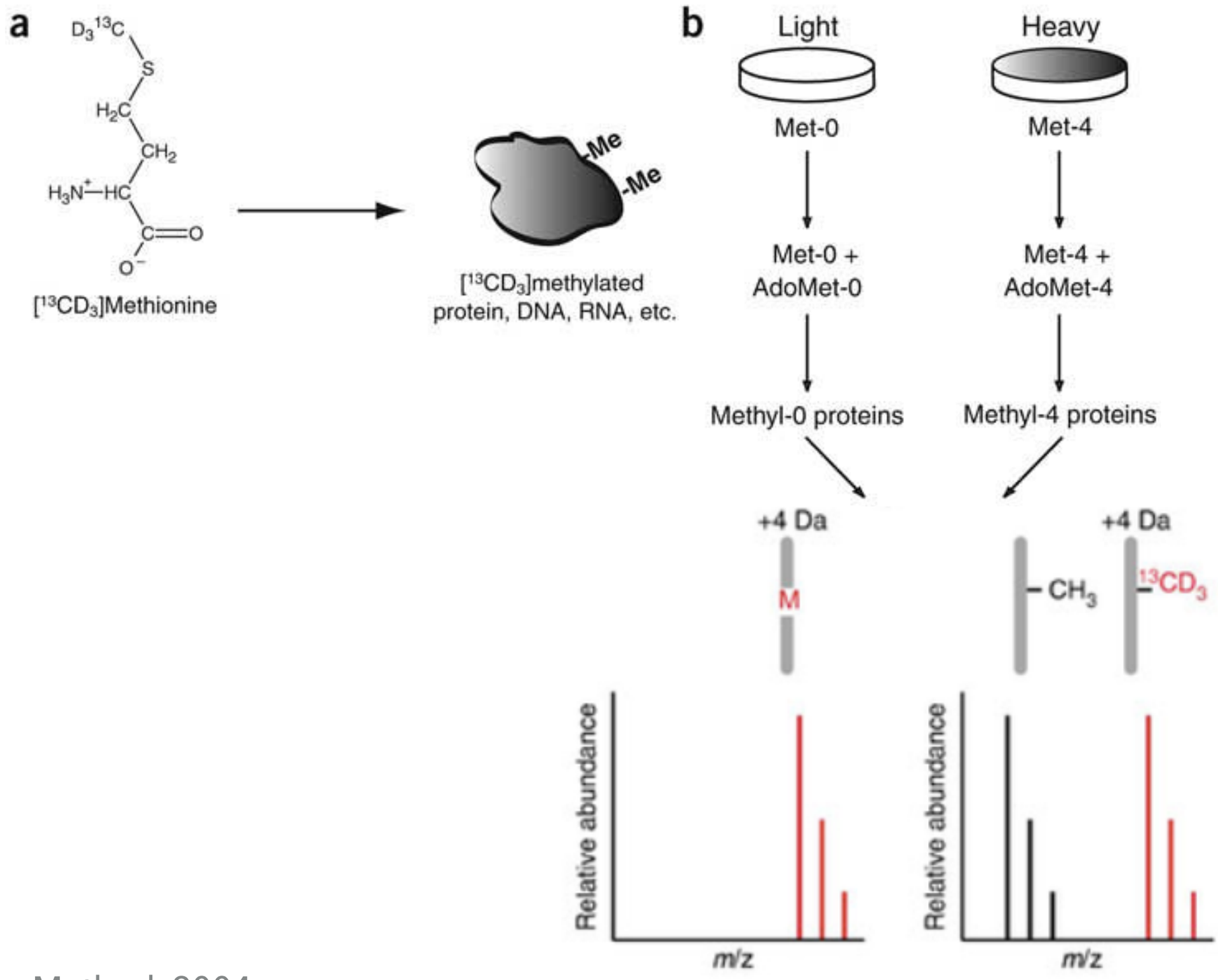
Multiplexed SILAC



Ong et al, Nat Chem Bio, 2005

What if you want to measure Post-Translational Modification?

Heavy Methyl SILAC



Ong et al, Nature Method, 2004

How does SILAC compare to iTRAQ?

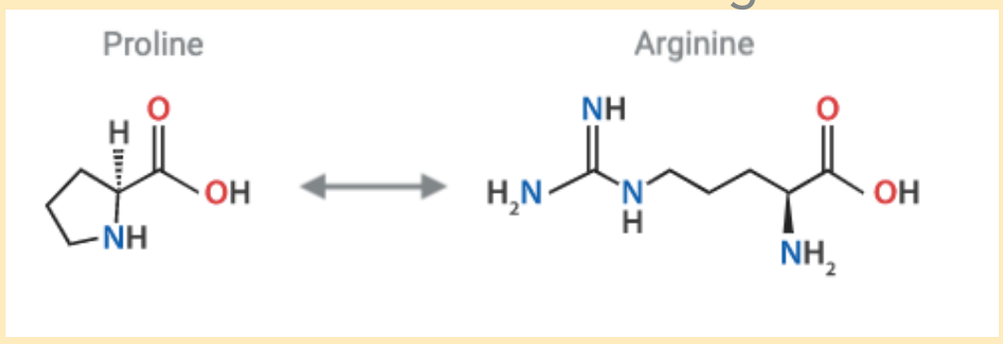
Advantage

accurate relative quantification

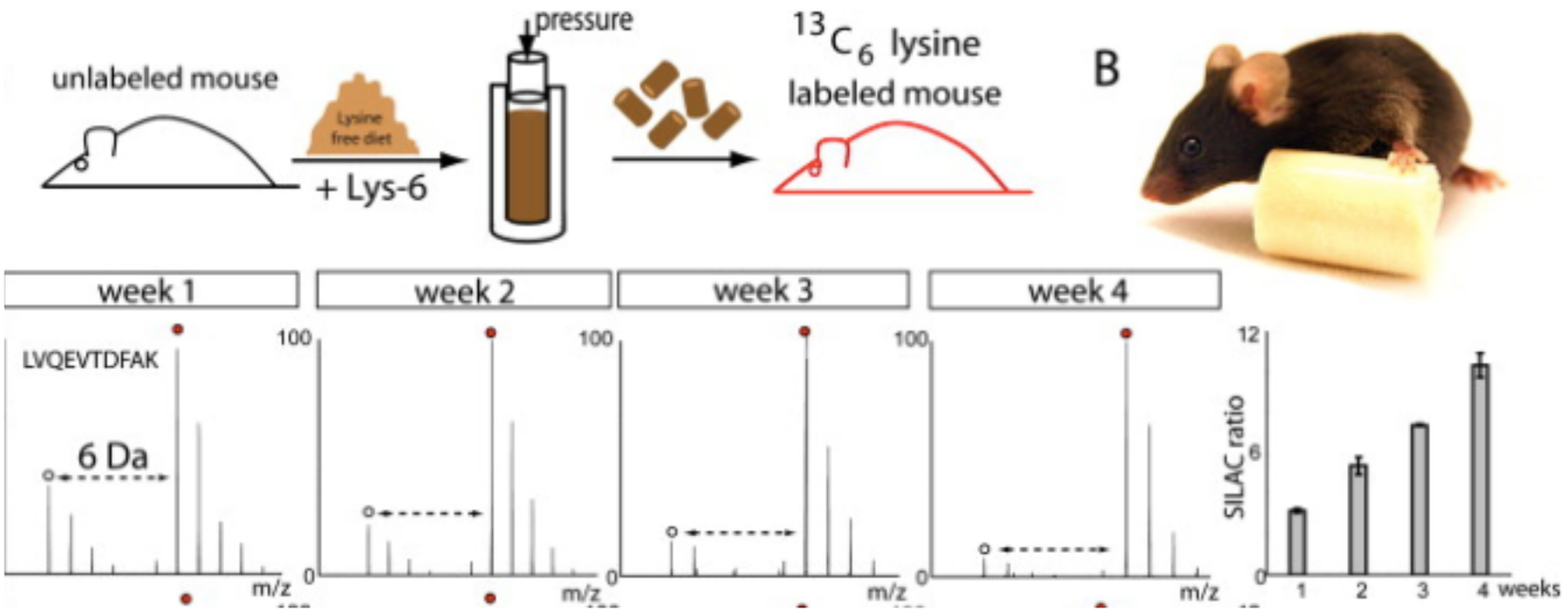
used on complex mixture of cells

Disadvantage

Amino Acid Interchange

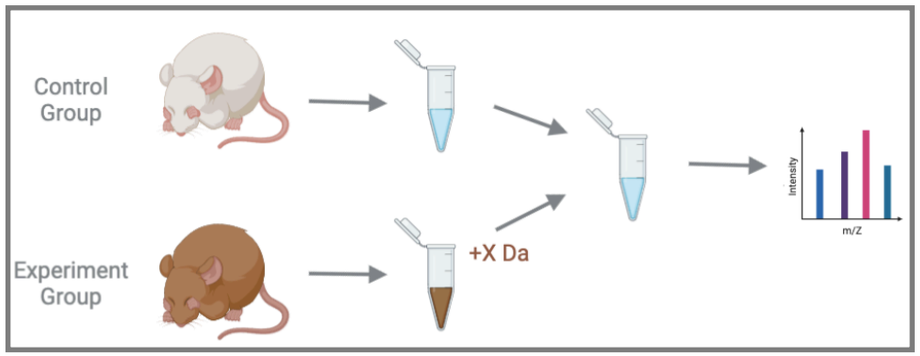


How to incorporate SILAC in my project?

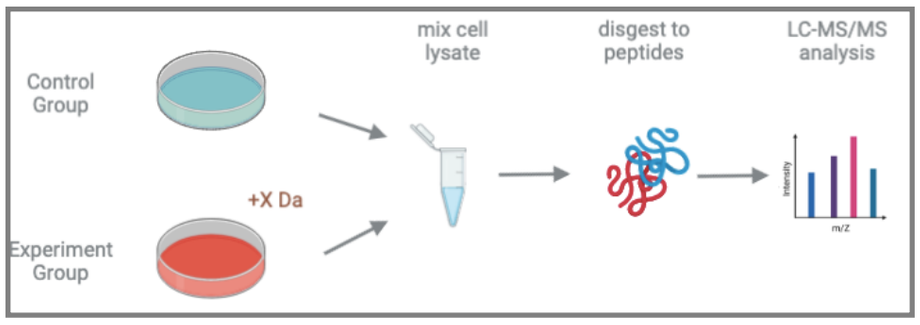


Kruger et al, 2008, Cell
Zanivan et al, 2011, Integrin and Cell Adhesion Mol.

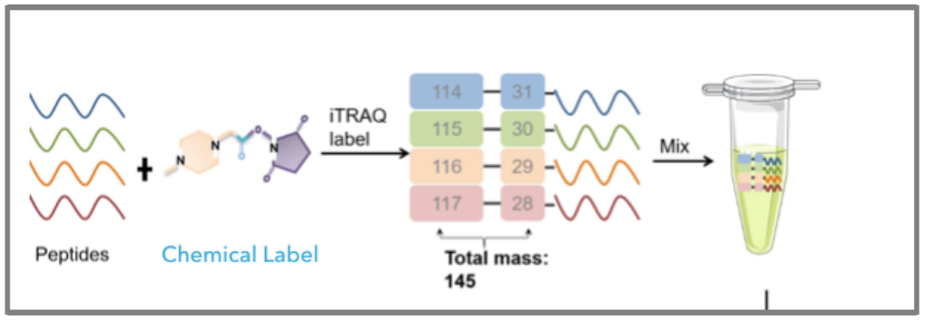
Summary



Metabolic labelling uses organism's own metabolism to label
Where as chemical labelling introduce a chemical tag on peptides

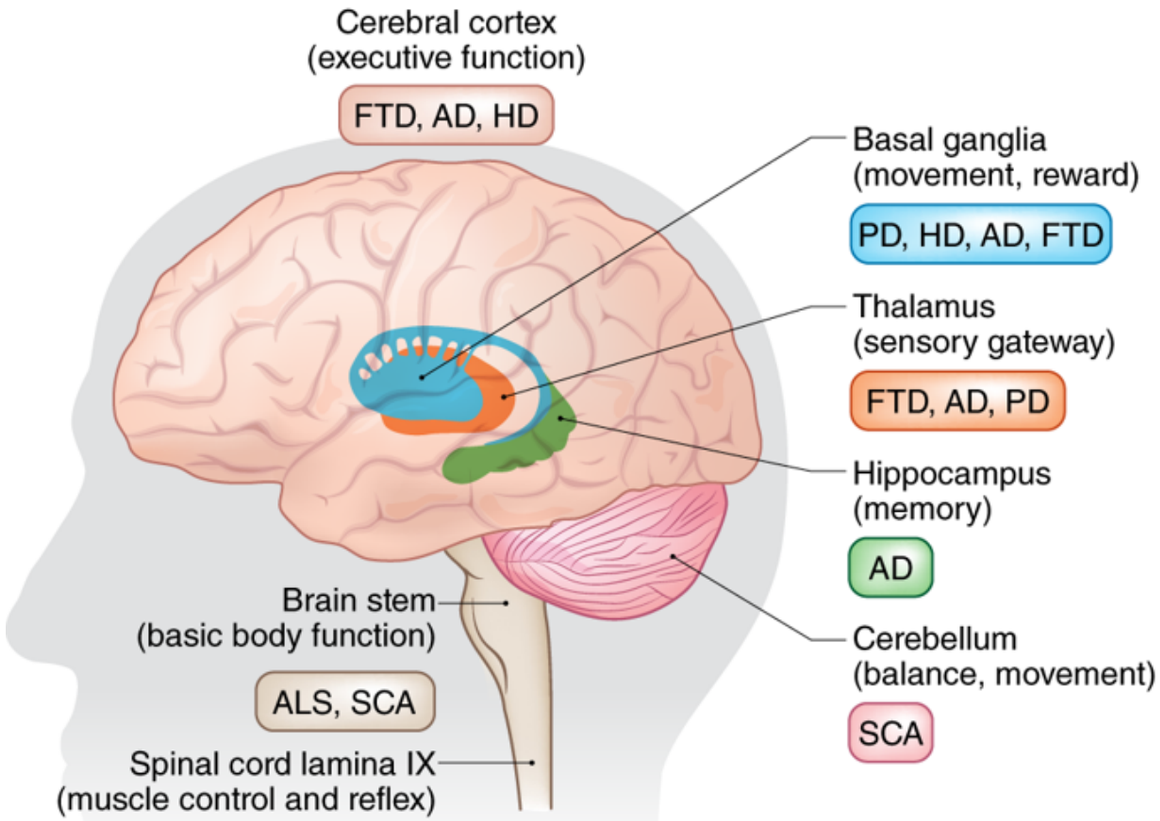


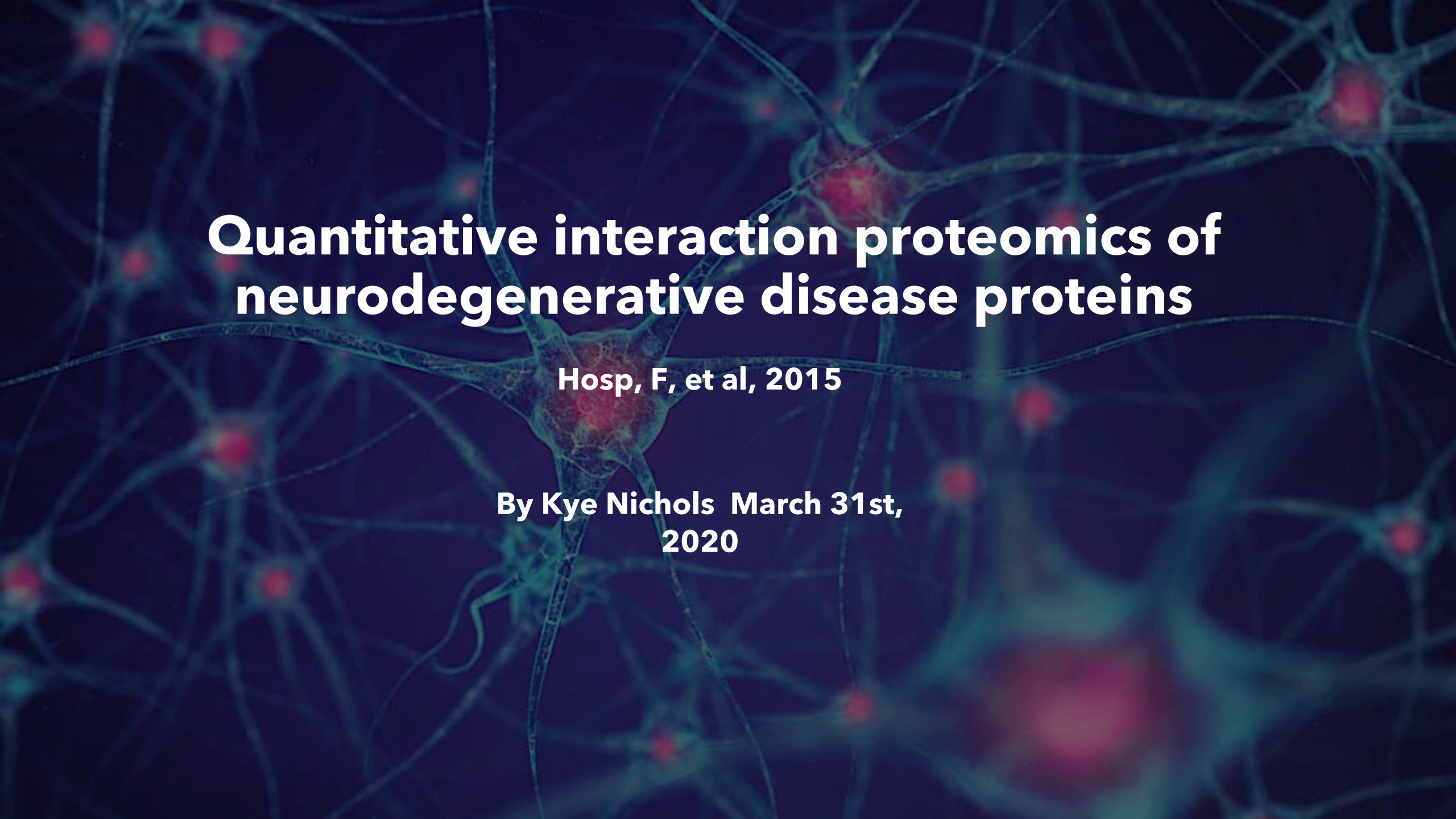
SILAC metabolically labels samples and determine protein relative abundance by Mass Spectrometry. SILAC measure methylation and can measure multiple trials.



iTRAQ and SILAC 's advantages and disadvantages in quantitative proteomics

How do we use SILAC to study neurodegenerative disorders?



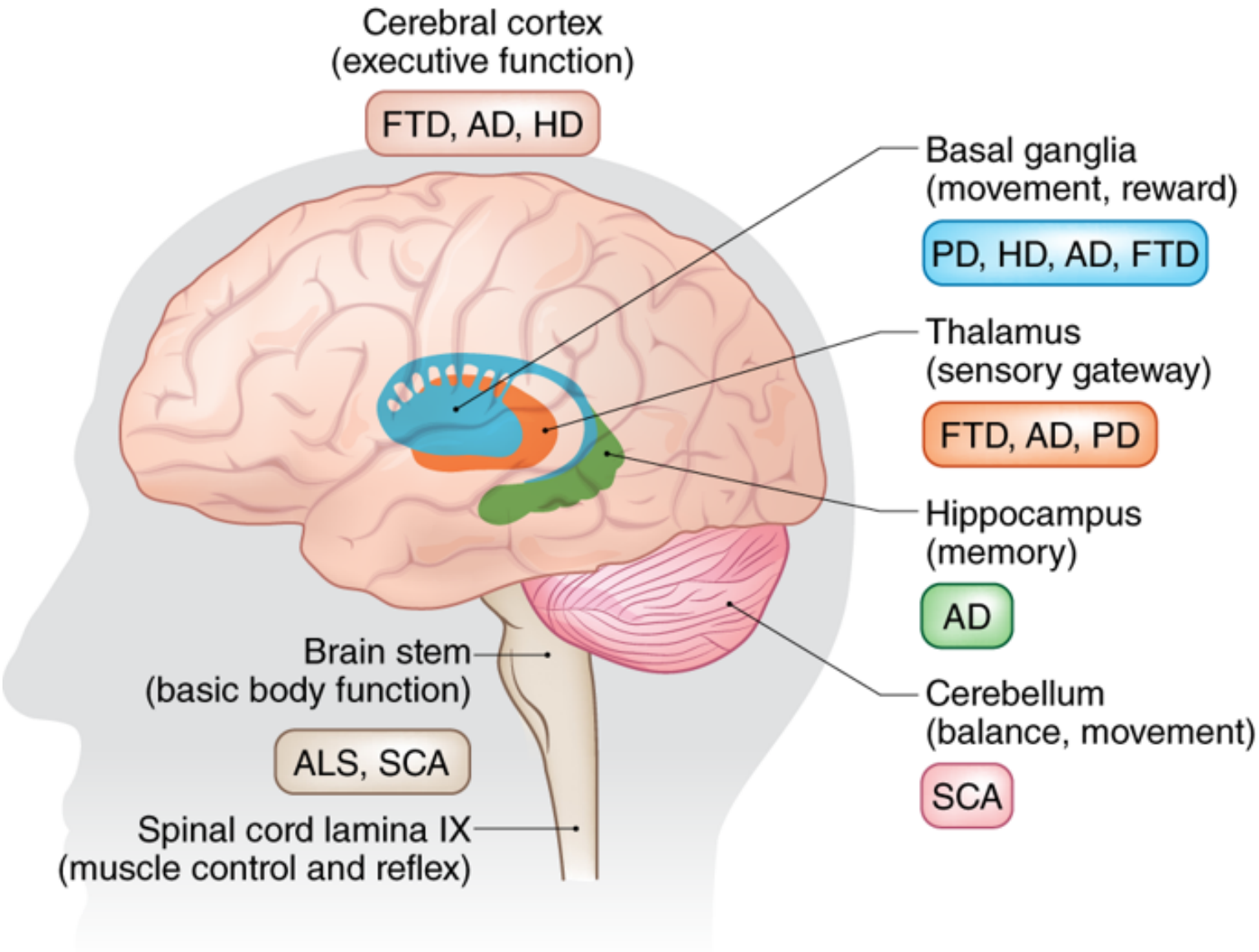


Quantitative interaction proteomics of neurodegenerative disease proteins

Hosp, F, et al, 2015

By Kye Nichols March 31st, 2020

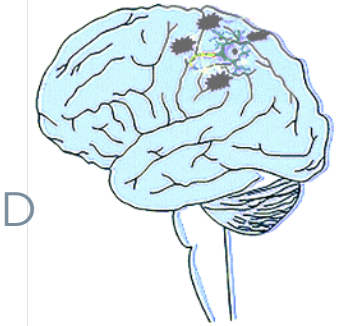
What are some examples of neurogenerative disorders?



Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD), Spinocerebellar Ataxia Type 1 (SCA1)

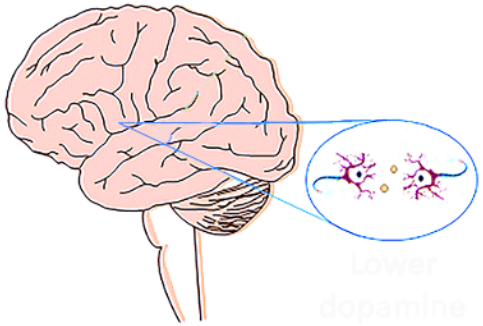
What are symptoms found in these neurodegenerative disorders?

AD



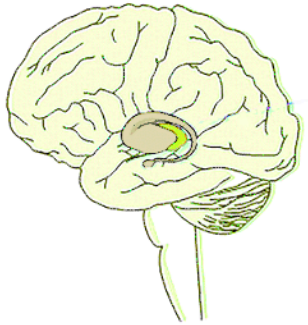
Memory Loss
Speech impairment

PD



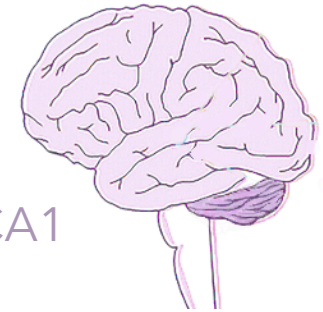
Involuntary movement
Balance

HD



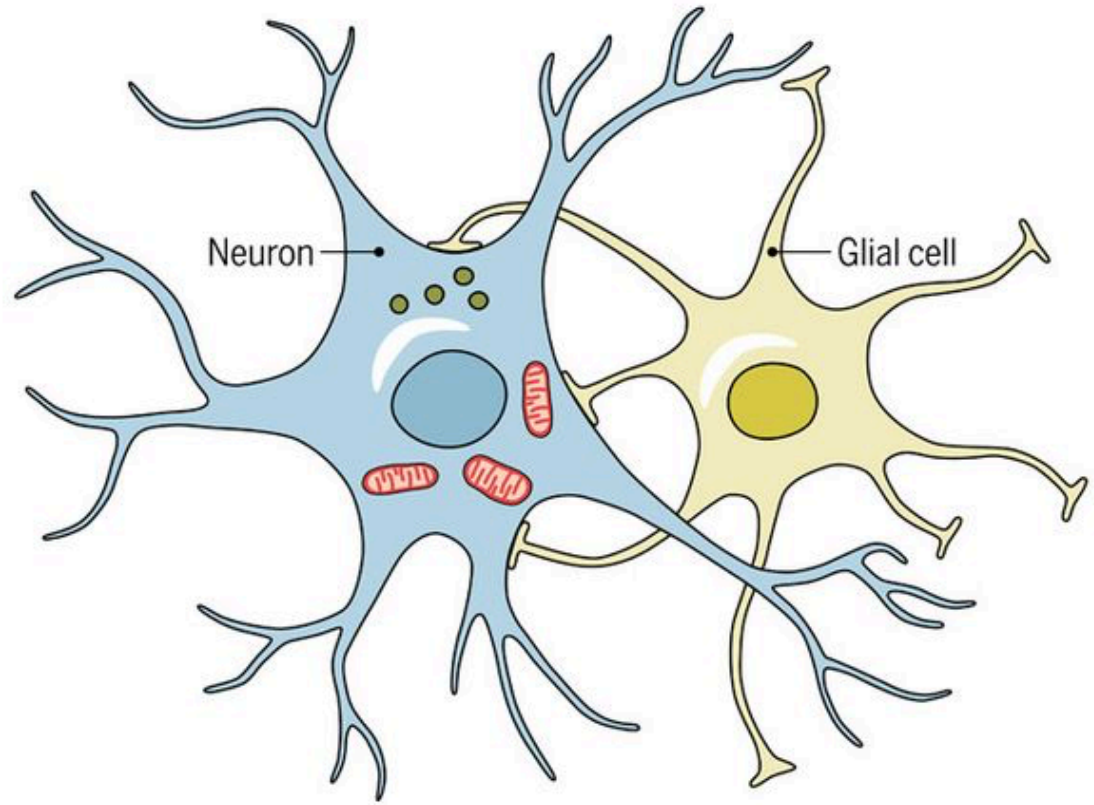
Depression
Writing changes

SCA1



Eye Movement
Spasticity

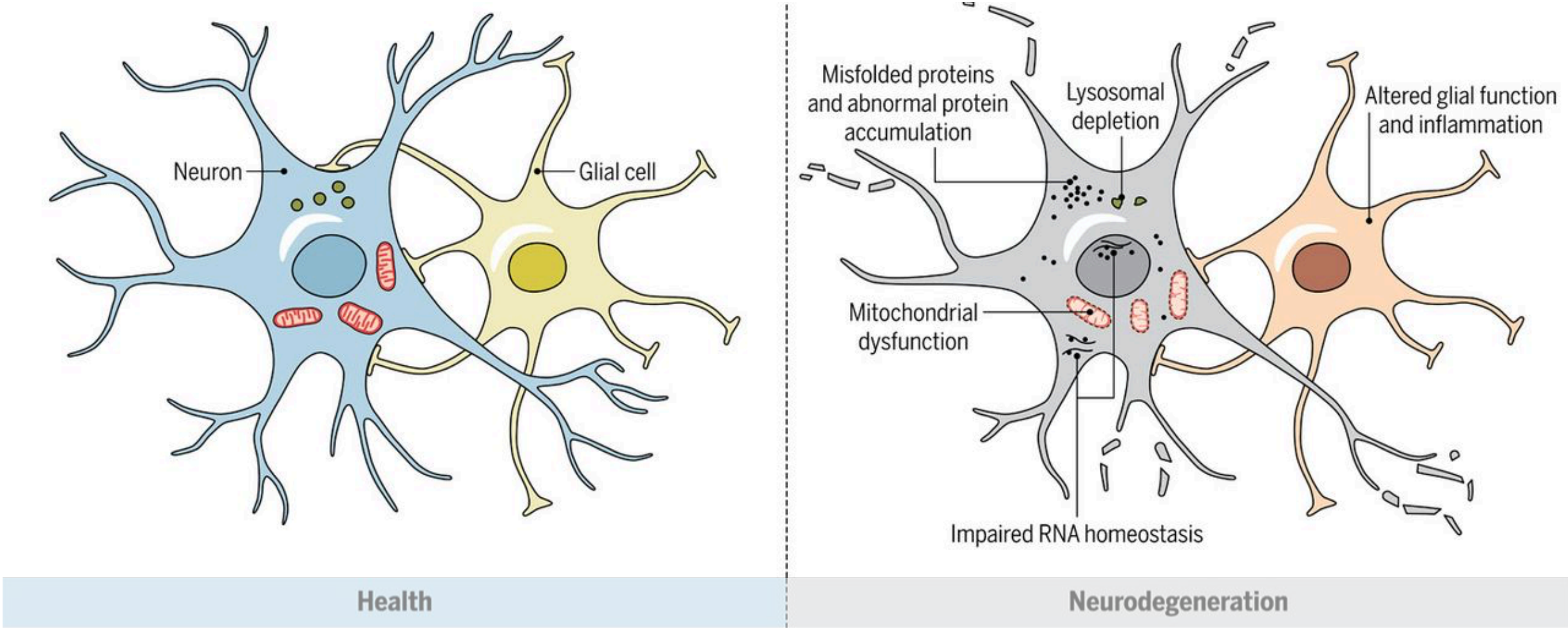
Why was qualitative proteomics used to study neurodegenerative diseases?



Health

Proteostasis is important for healthy neuronal function

What are some molecular and cellular hallmarks of neurodegenerative diseases?



Changes in proteostasis can cause cause *neurodegeneration*

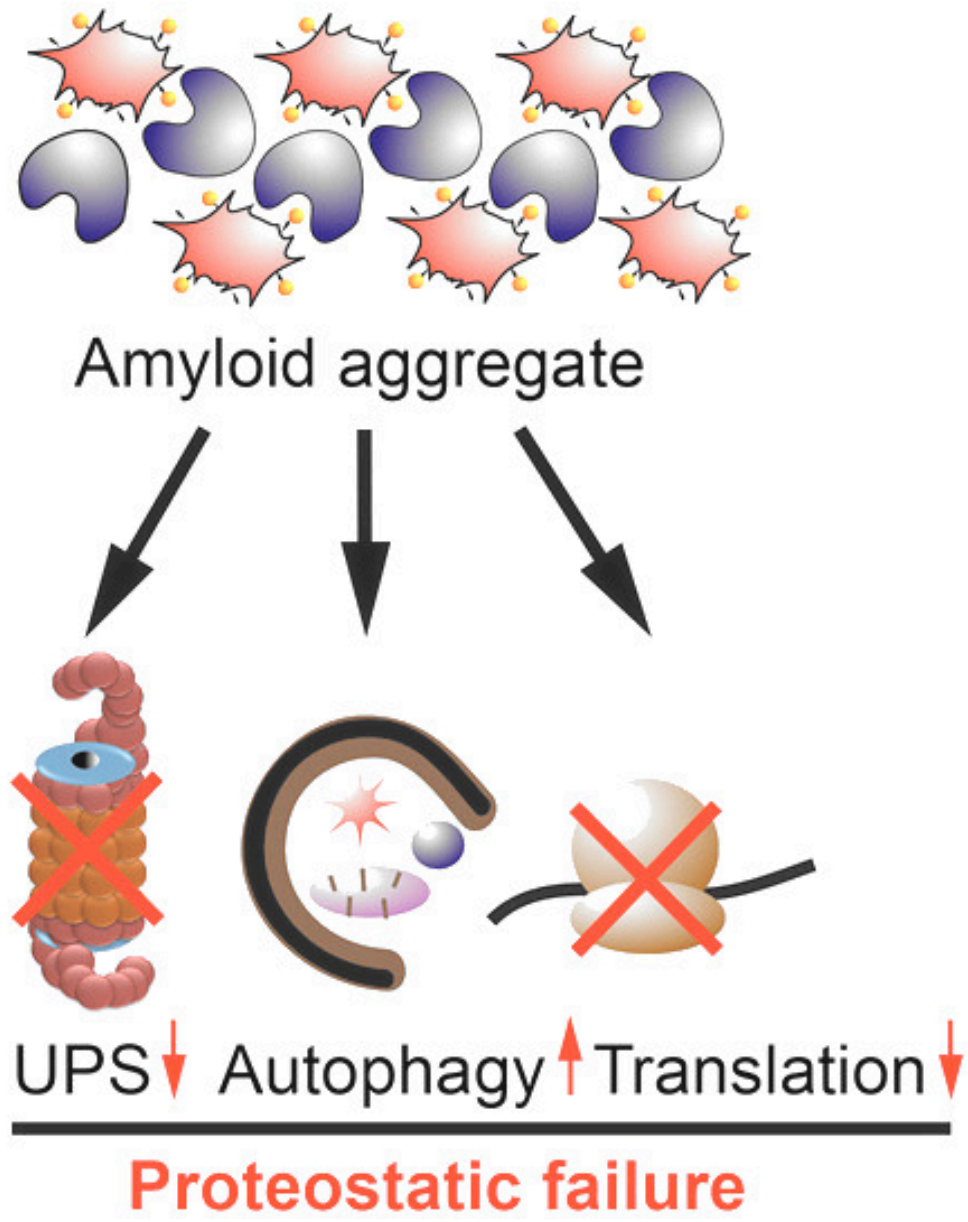
What proteins are linked to neurodegeneration?

AD — APP, PSEN1/2

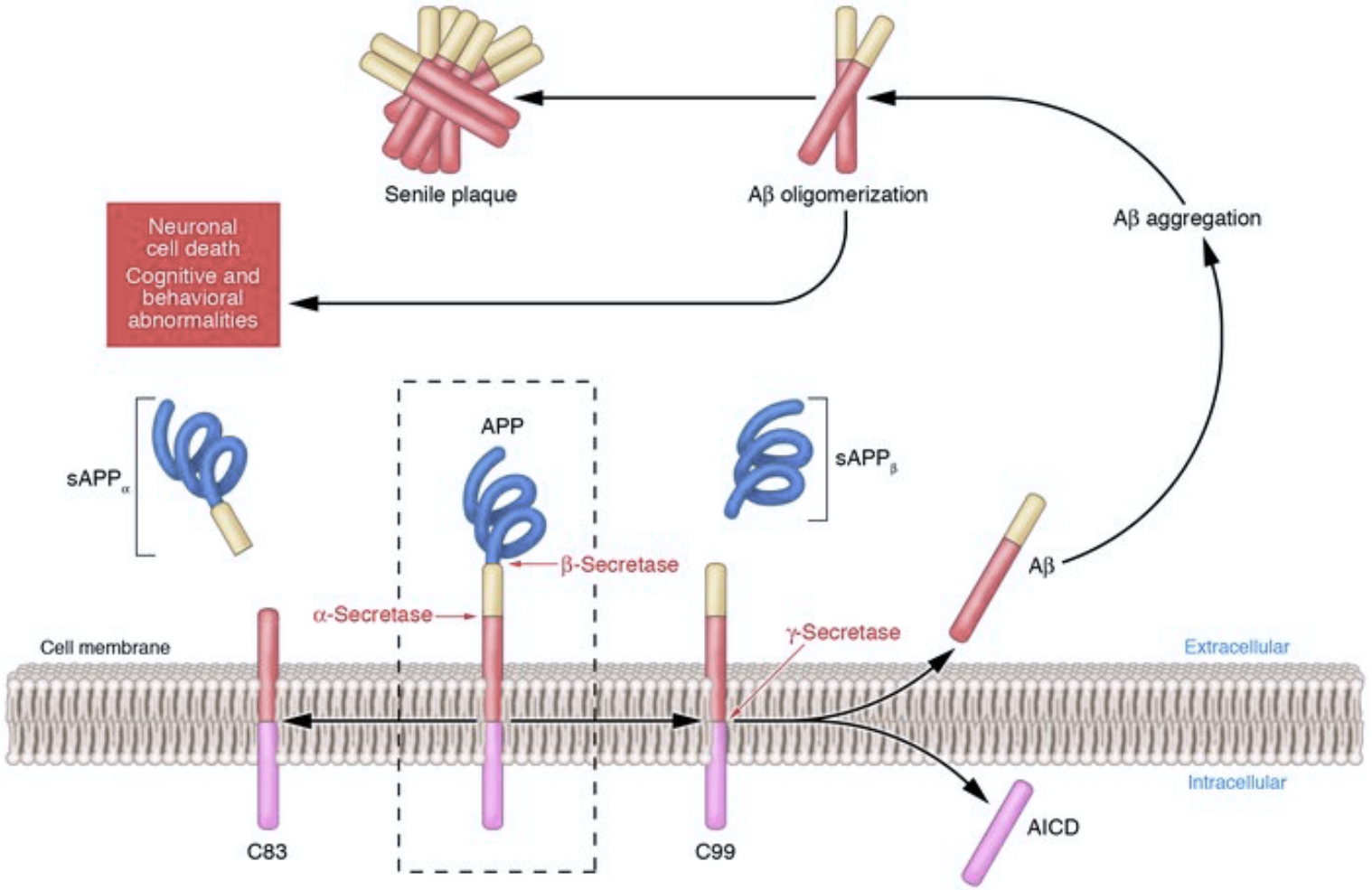
PD — PARK2

HD — HTT

SCA1 — ATXN1

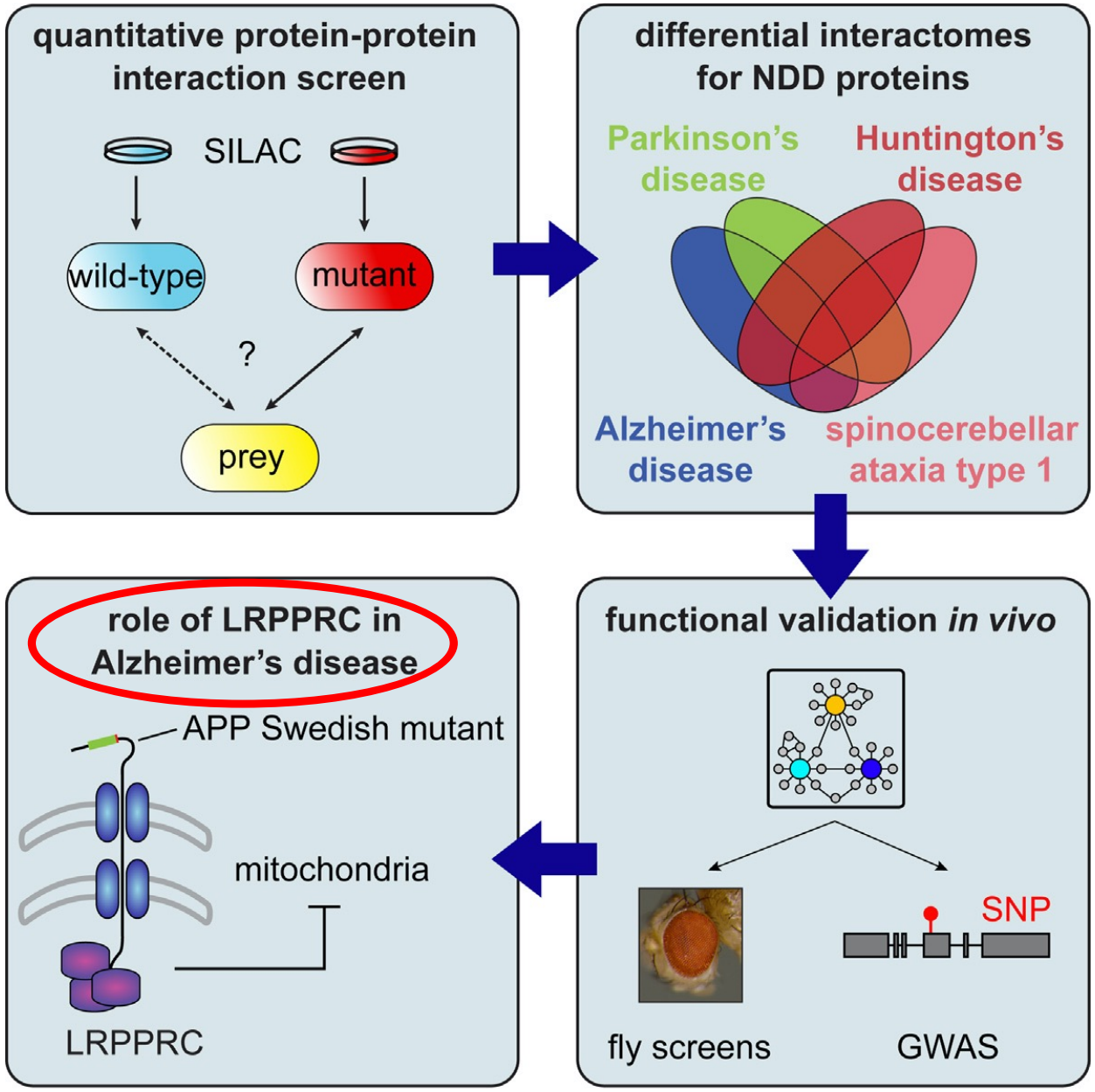


What is the GAP in knowledge?



Construction of disease-associated protein interaction networks is a major challenge

What are common and unique proteins associated with neurodegeneration?



Why is SILAC effective for studying *neurodegeneration*?

Y2H and AP-MS are "semiquantative"

SILAC can differentiate

contaminants

&

specific binding partners

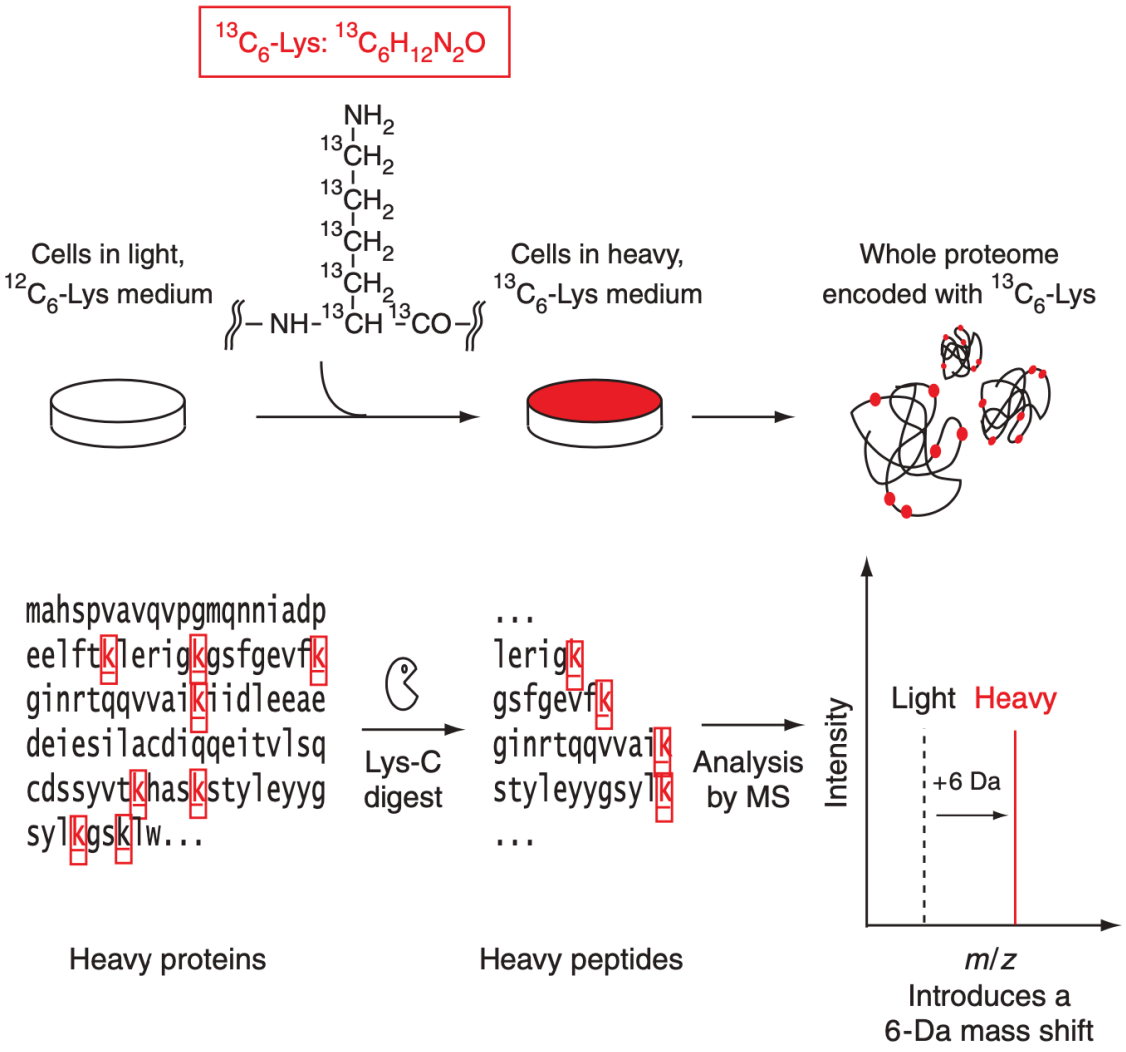
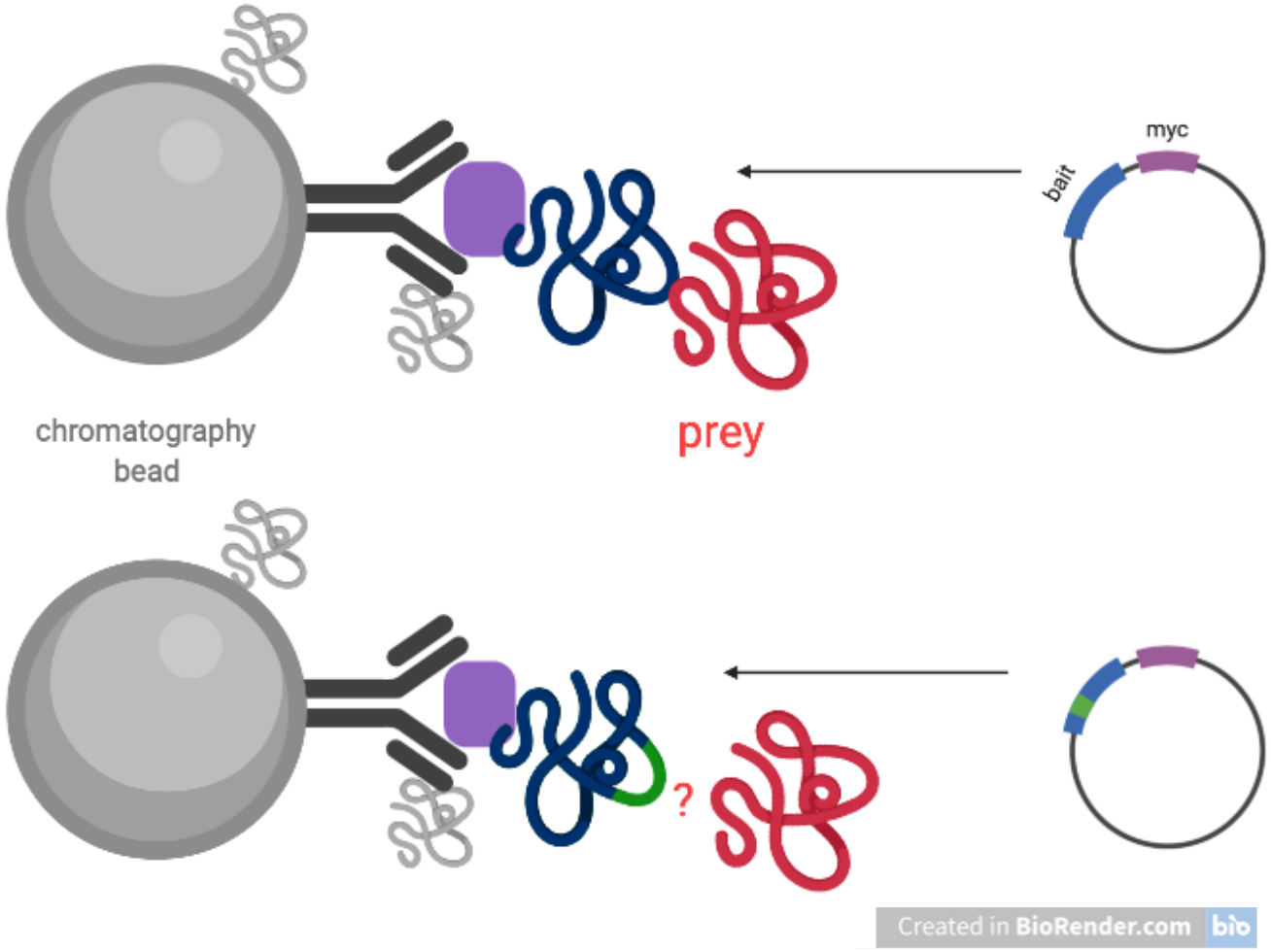
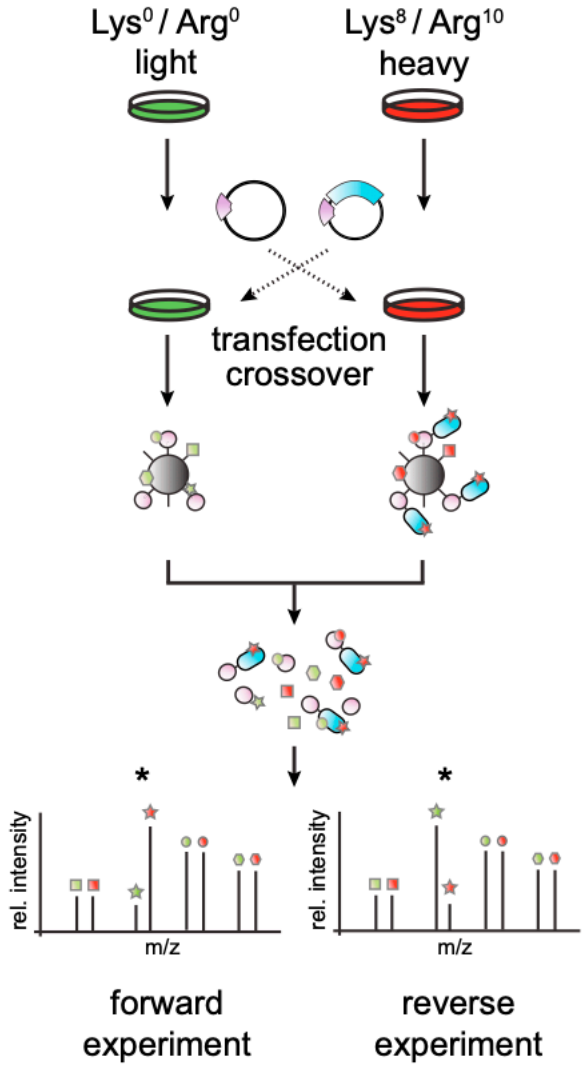


Fig. 1A: How was SILAC used to identify unique proteins associated with *neurodegeneration*?





SILAC

transient transfection

expression of constructs

affinity purification

combine 1:1 and elute

MS sample preparation

LC-MS/MS analysis (LTQ-Orbitrap)

Fig. 1D: What are unique and shared interactions between diseases according to online databases?

Enriched GO Terms

P values

GO biological process complete	Caenorhabditis elegans (REF) #	Client Text Box Input #	expected	Fold Enrichment	+/-	P value
positive regulation of transcription from RNA polymerase II promoter involved in unfolded protein response	4	4	.01	> 5	+	1.38E-06
positive regulation of transcription from RNA polymerase II promoter involved in cellular response to chemical stimulus	4	4	.01	> 5	+	1.38E-06
mitochondrial unfolded protein response	7	7	.02	> 5	+	5.26E-13
response to unfolded protein	69	47	.22	> 5	+	4.03E-98
cellular response to unfolded protein	69	47	.22	> 5	+	4.03E-98
endoplasmic reticulum unfolded protein response	64	43	.20	> 5	+	2.99E-88
cellular response to topologically incorrect protein	74	47	.23	> 5	+	1.08E-96
ERAD pathway	8	5	.03	> 5	+	1.04E-07
ER-associated ubiquitin-dependent protein catabolic process	8	5	.03	> 5	+	1.04E-07
response to topologically incorrect protein	80	48	.25	> 5	+	6.14E-98
response to endoplasmic reticulum stress	77	45	.24	> 5	+	2.78E-90
response to misfolded protein	9	3	.03	> 5	+	5.13E-03
proteasome-mediated ubiquitin-dependent protein catabolic process	22	5	.07	> 5	+	1.58E-05
proteasomal protein catabolic process	22	5	.07	> 5	+	1.58E-05
response to heat	52	11	.16	> 5	+	3.14E-14

PANTHER Enrichment Analysis Results
(http://pantherdb.org)

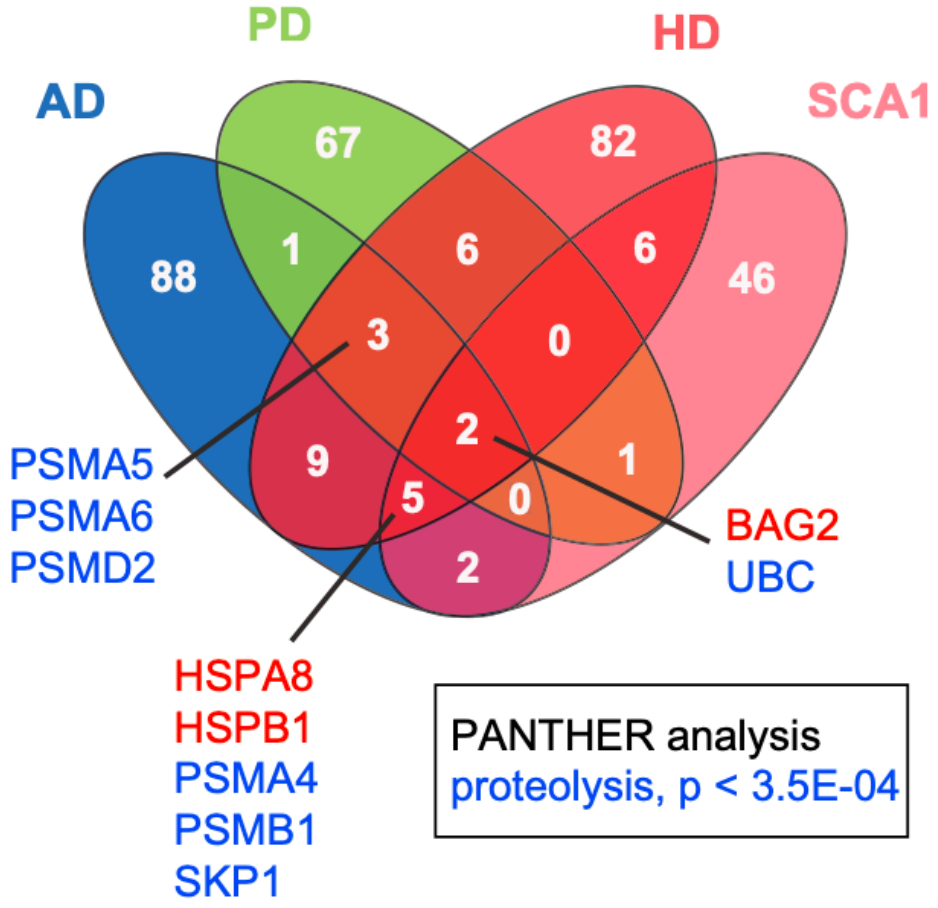
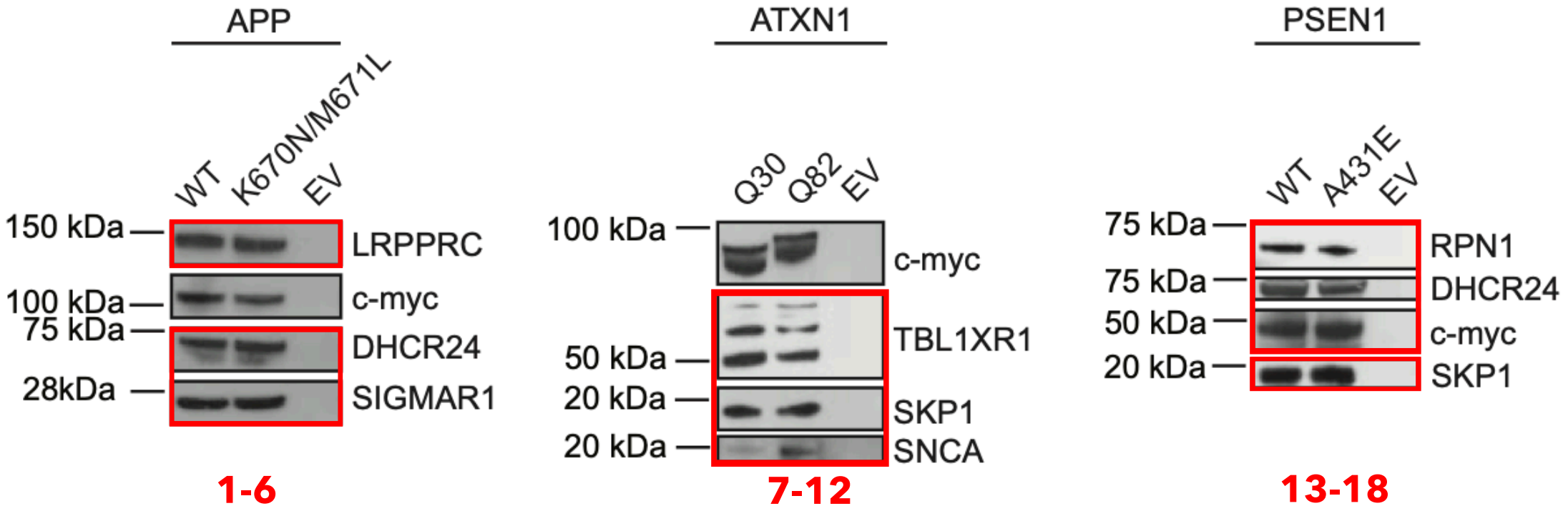
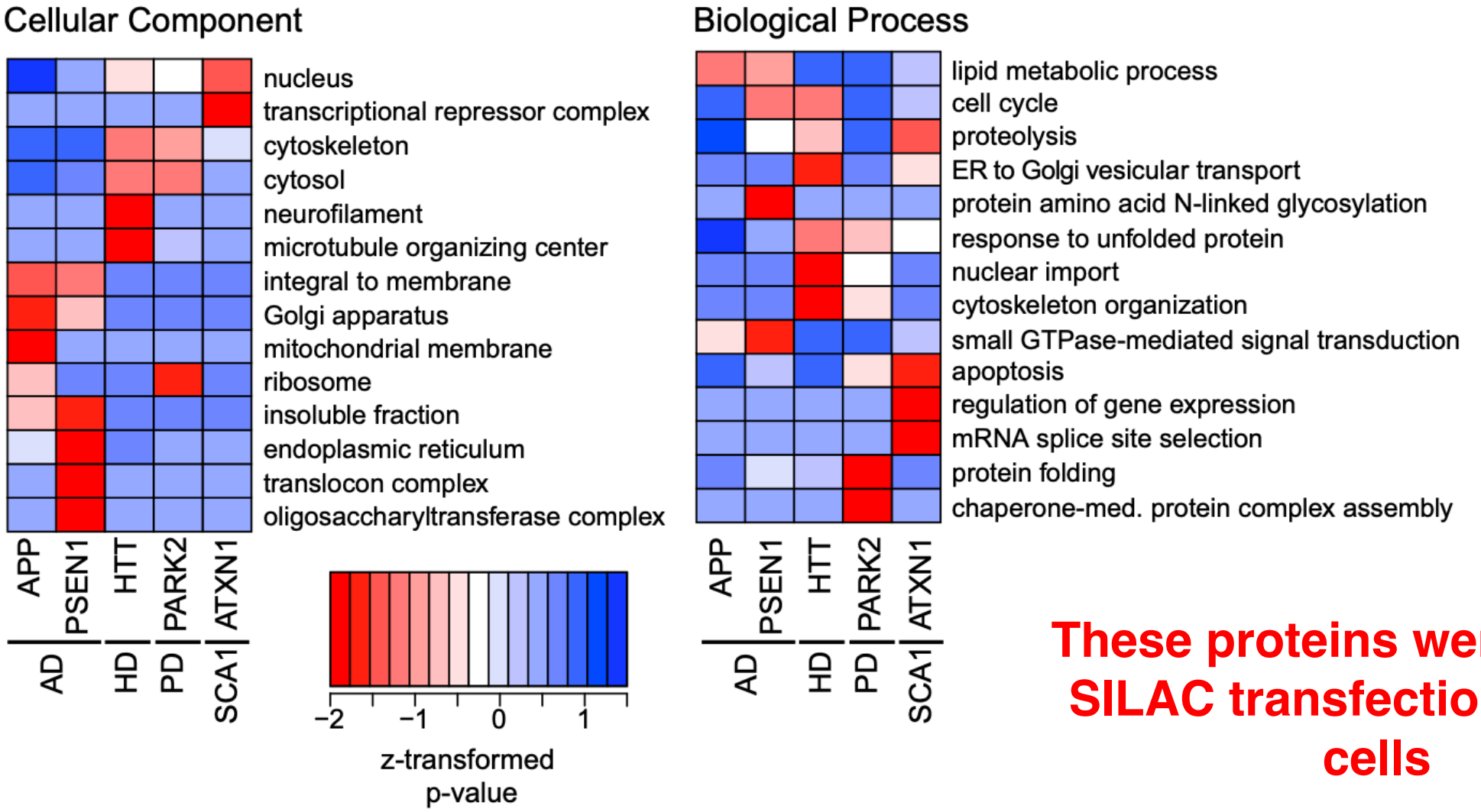


Fig 1D: What binding partners were verified in protein interaction screening?



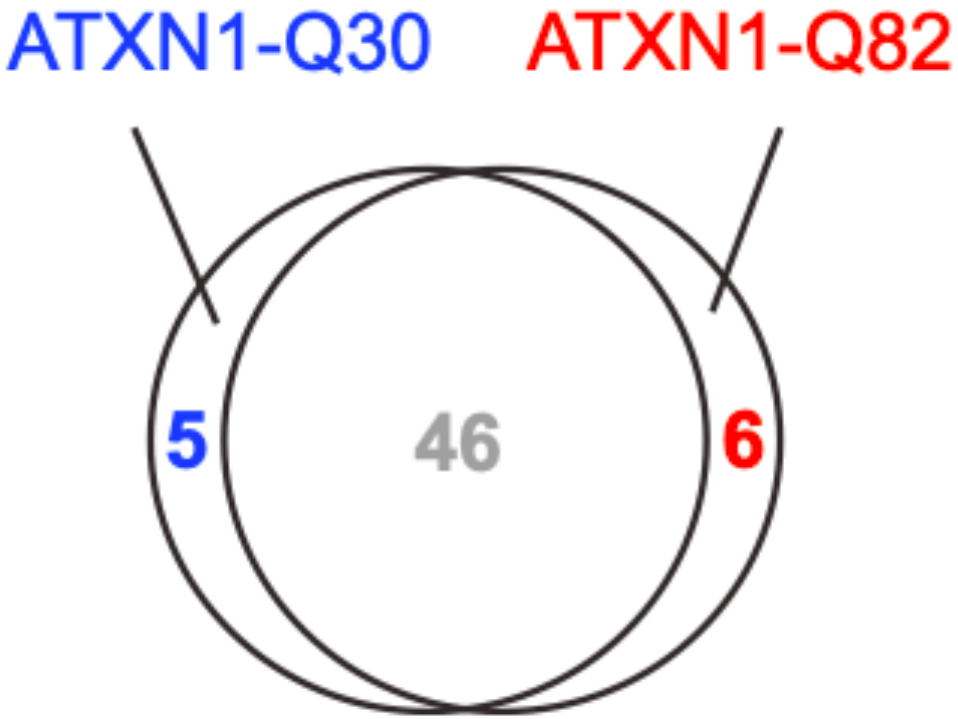
Evidence of 18 interactions by co-IP in transfected HEK cells

Fig. 1E: What cellular processes are bait proteins involved in?



These proteins were bait for SILAC transfection of HEK cells

Fig. 1B: How was SILAC used to identify preferential binding to Ataxin-1?



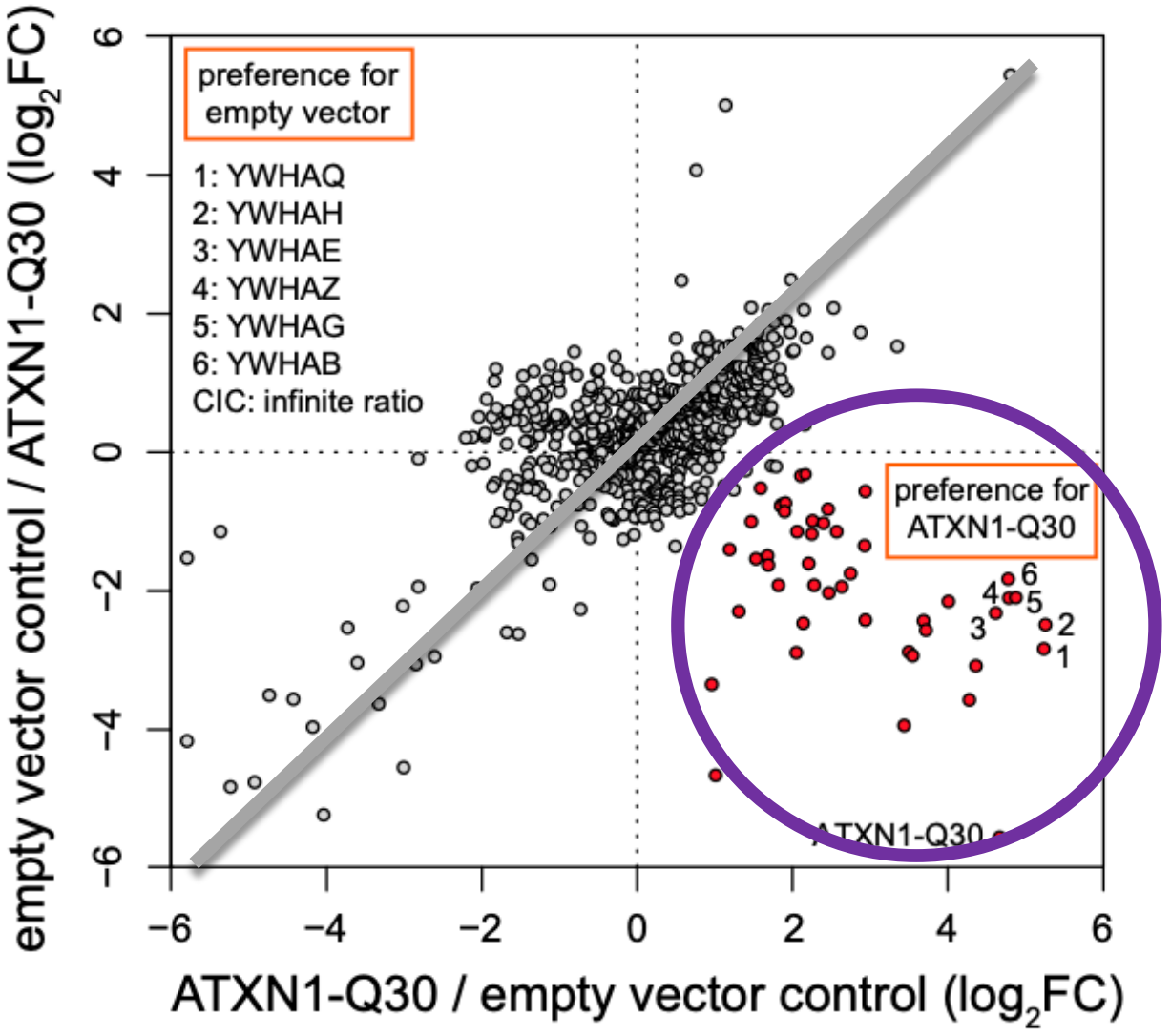
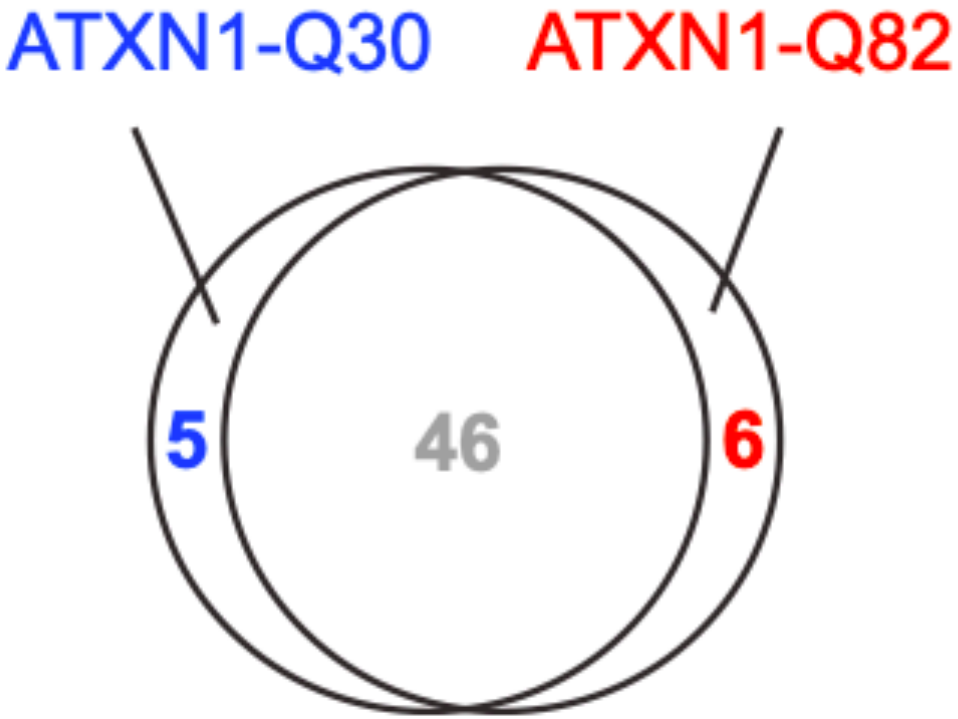


Fig. 1C: How was SILAC used to identify preferential binding to Ataxin-1 mutants?



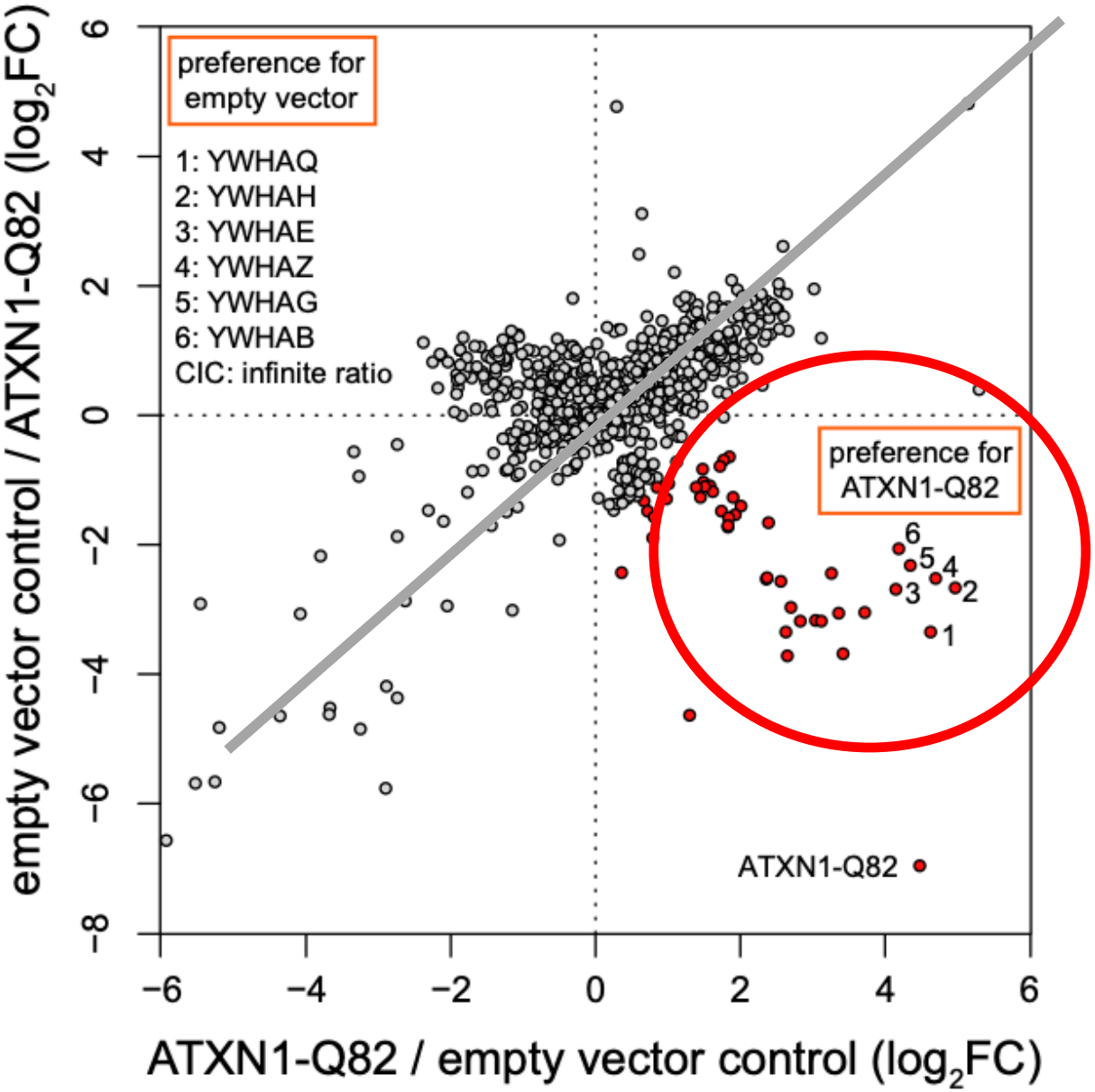
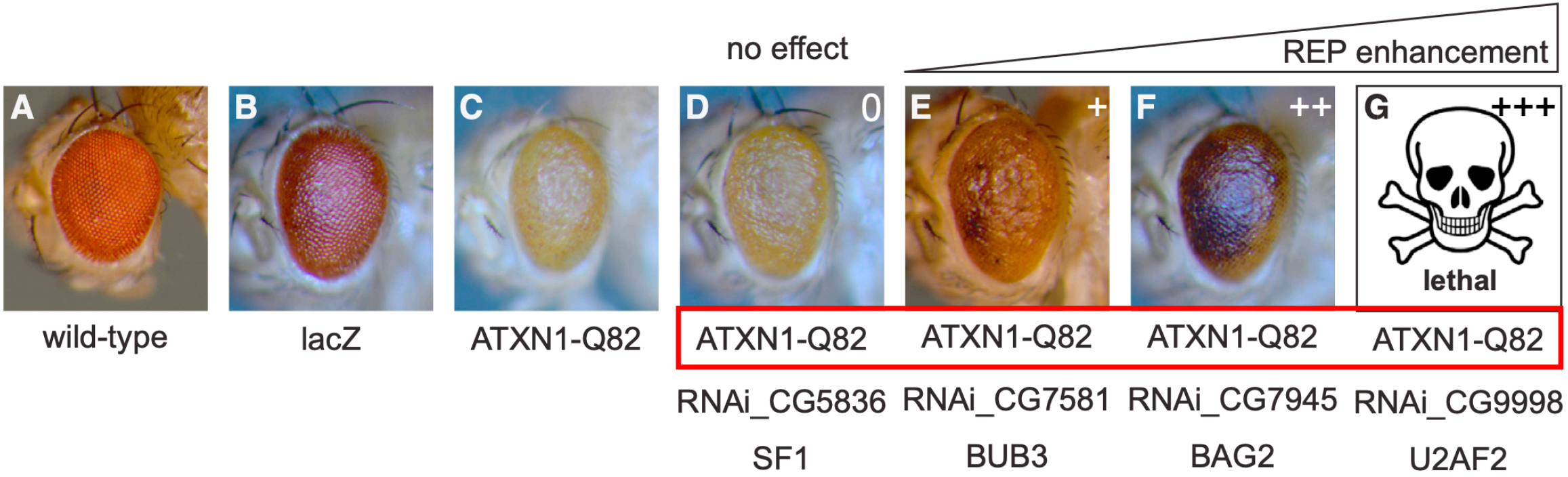


Fig. 2: How is neurodegeneration affected by Ataxin-1 mutations in Drosophila models?



RNA silenced ATXN1 forms induced Rough-Eye Phenotype(REP) *signifying NDD*

Fig. 3A: How does Gene Set Enrichment compare with quantitative proteomic analysis?

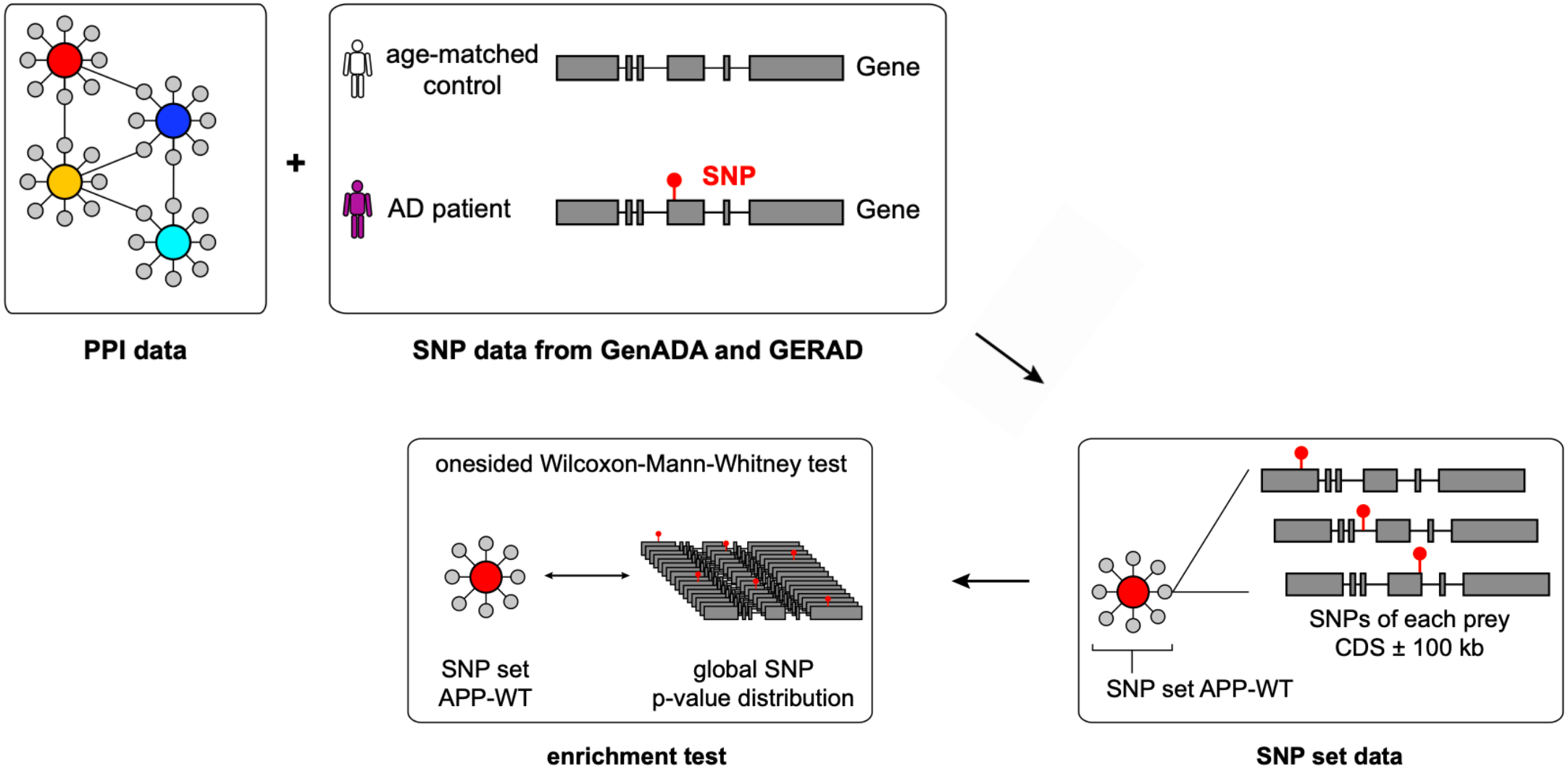
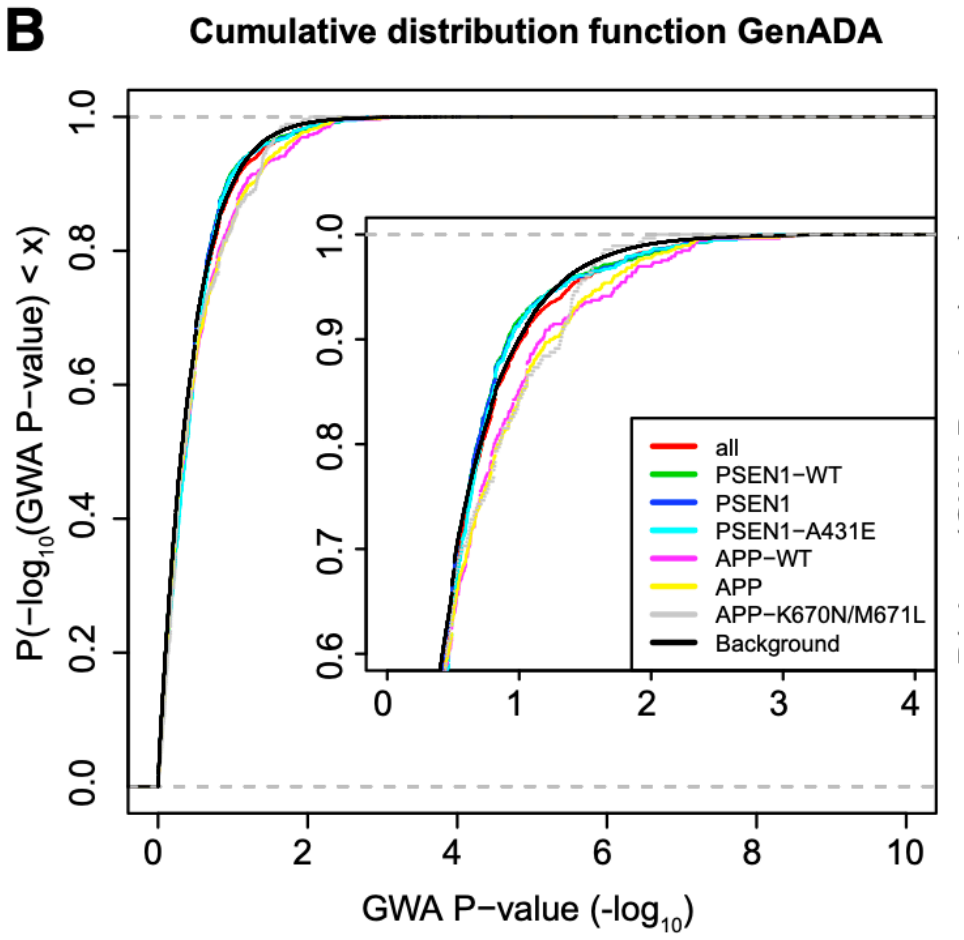


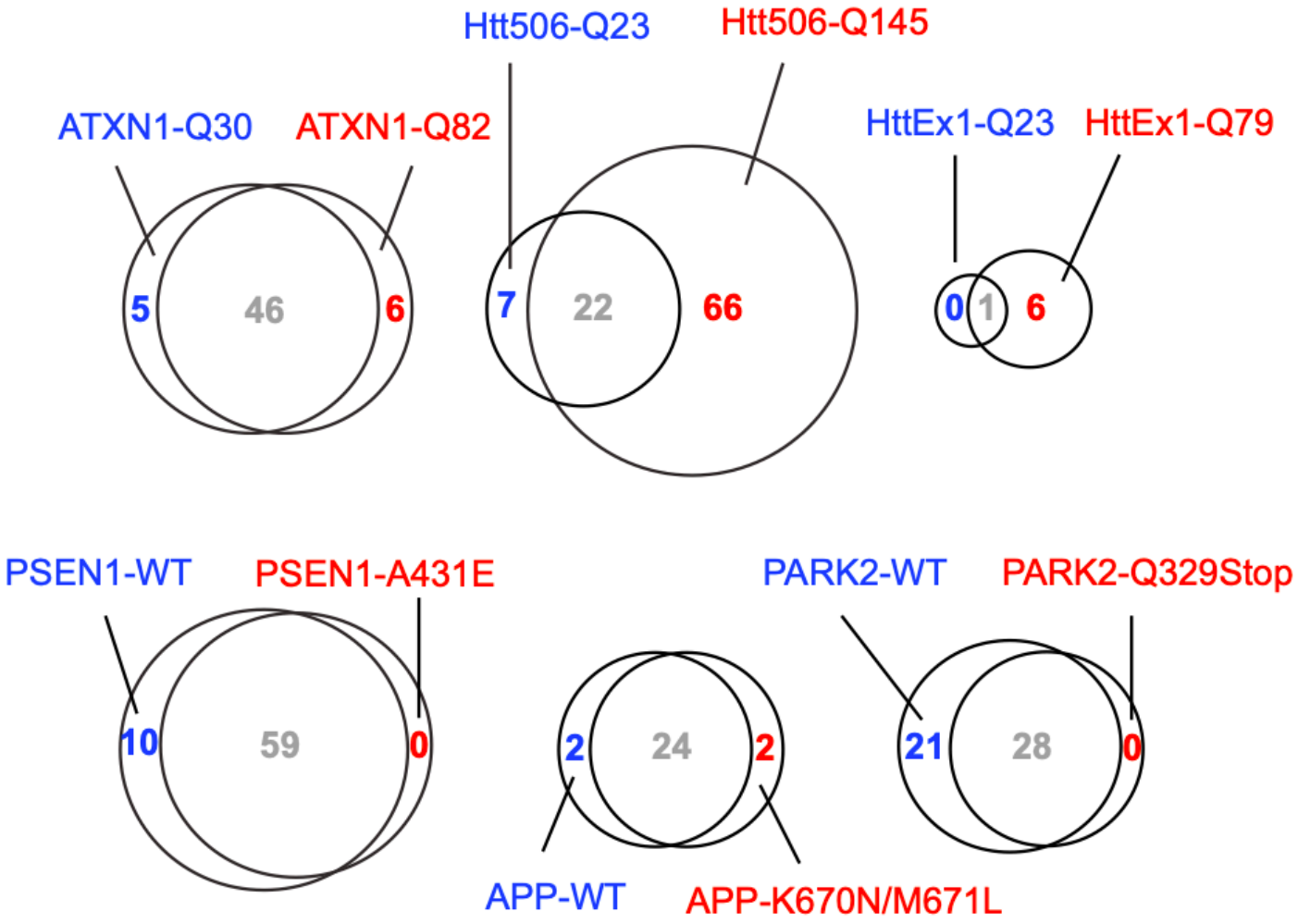
Fig. 3B: How does SNP enrichment signify NDD-association?



	GenADA + GERAD
SNP set	**p-value**
APP-WT	4.1E-05
APP-K670N/M671L	8.6E-07
APP combined	2.4E-08
PSEN1-WT	2.6E-01
PSEN1-A431E	9.9E-04
PSEN1 combined	6.2E-02
APP+PSEN1 combined	5.6E-05

Least significant enrichment of SNPs for WT

Fig. 4B: How did preferential binding behavior compare between disease-associated variants?

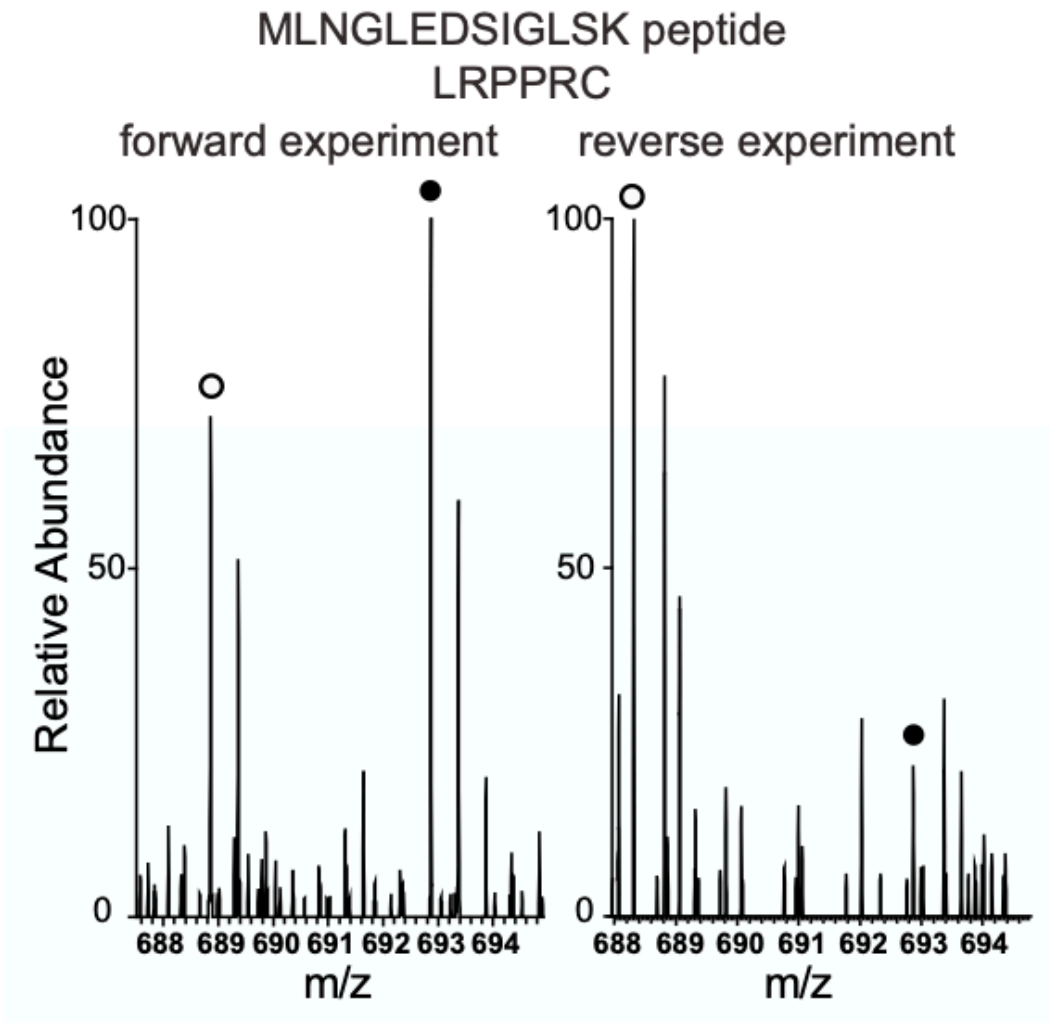


NDD-associated binding

WT-specific binding

Non-specific contaminant

Fig. 5A: How does “Swedish” variant interact compared to wildtype?



Swedish variant is a known motivator in early-onset AD

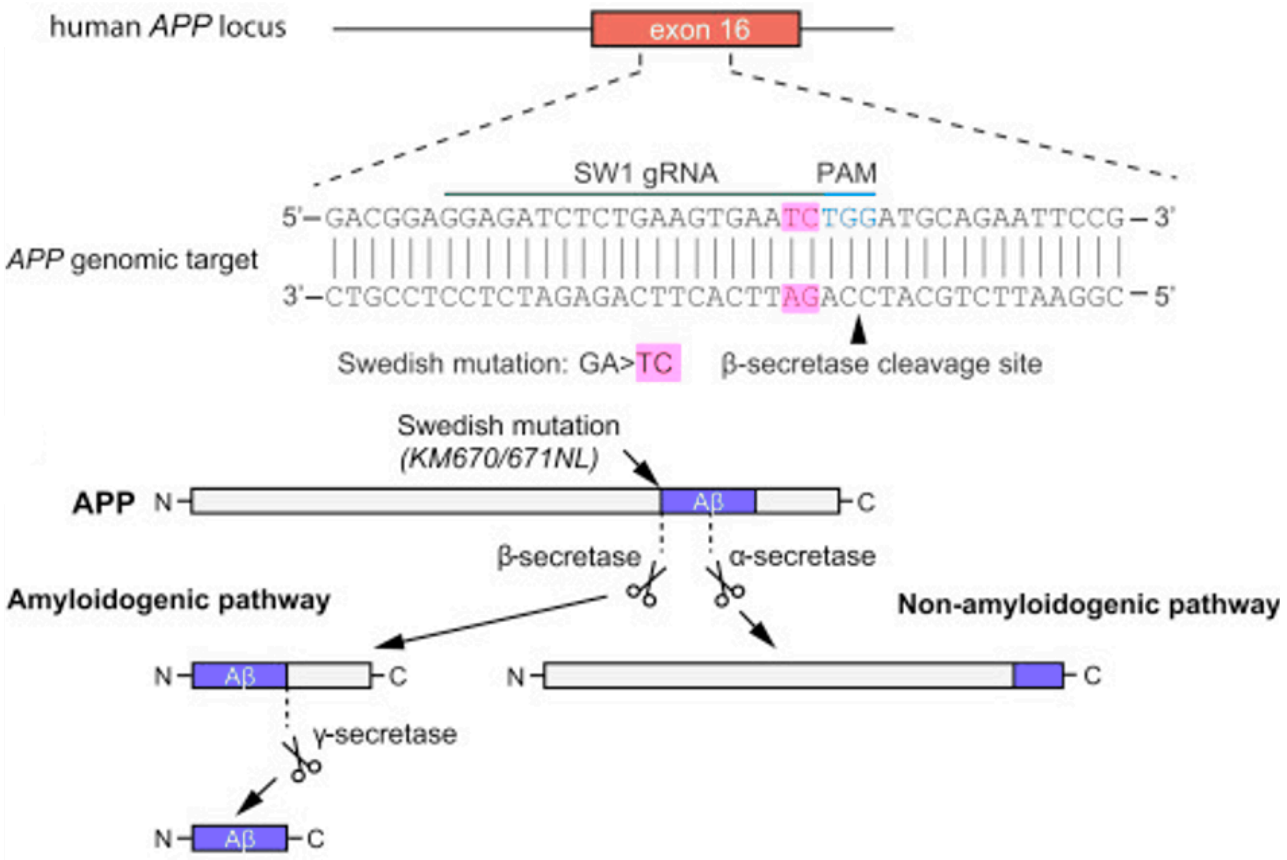
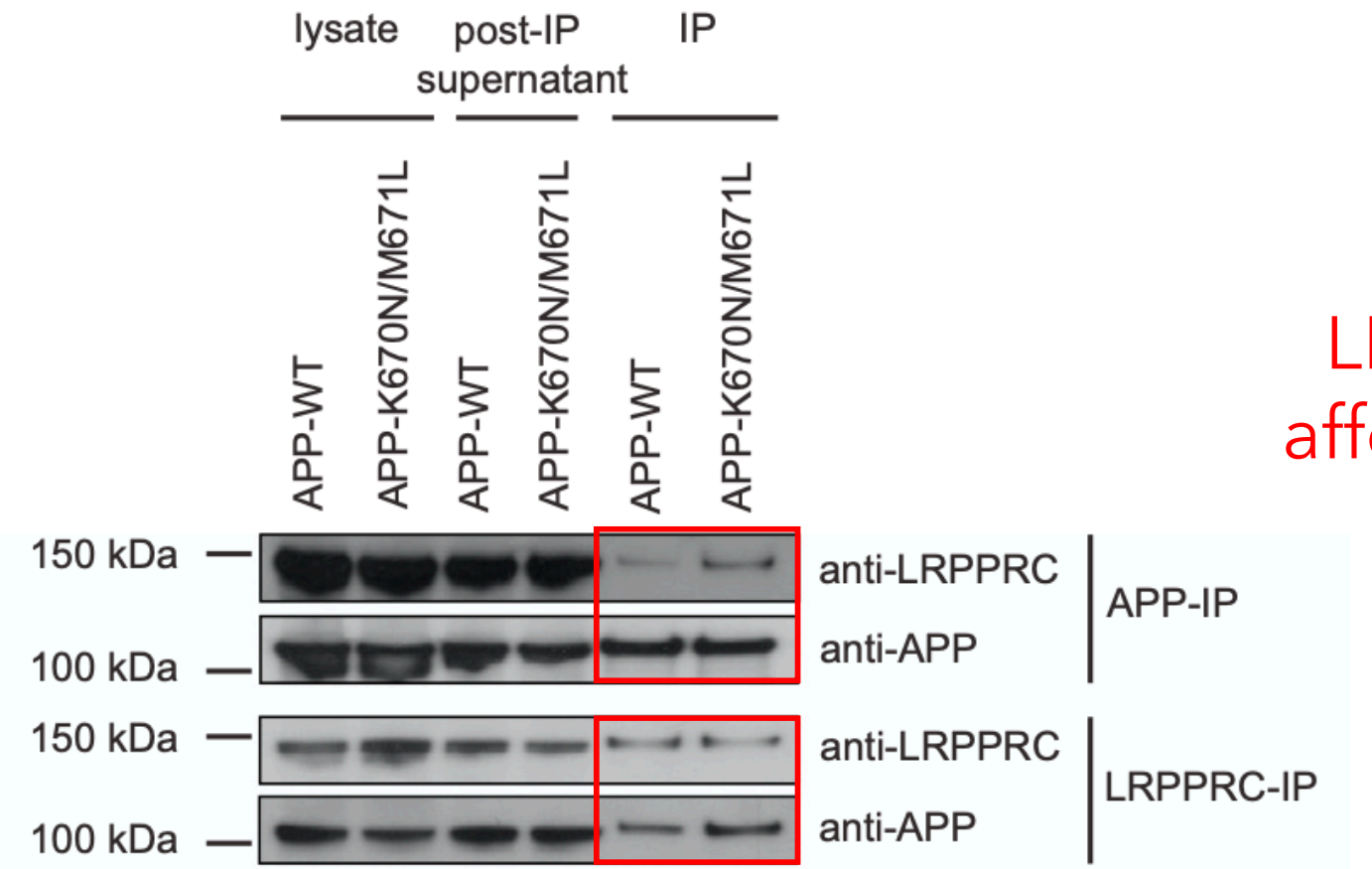


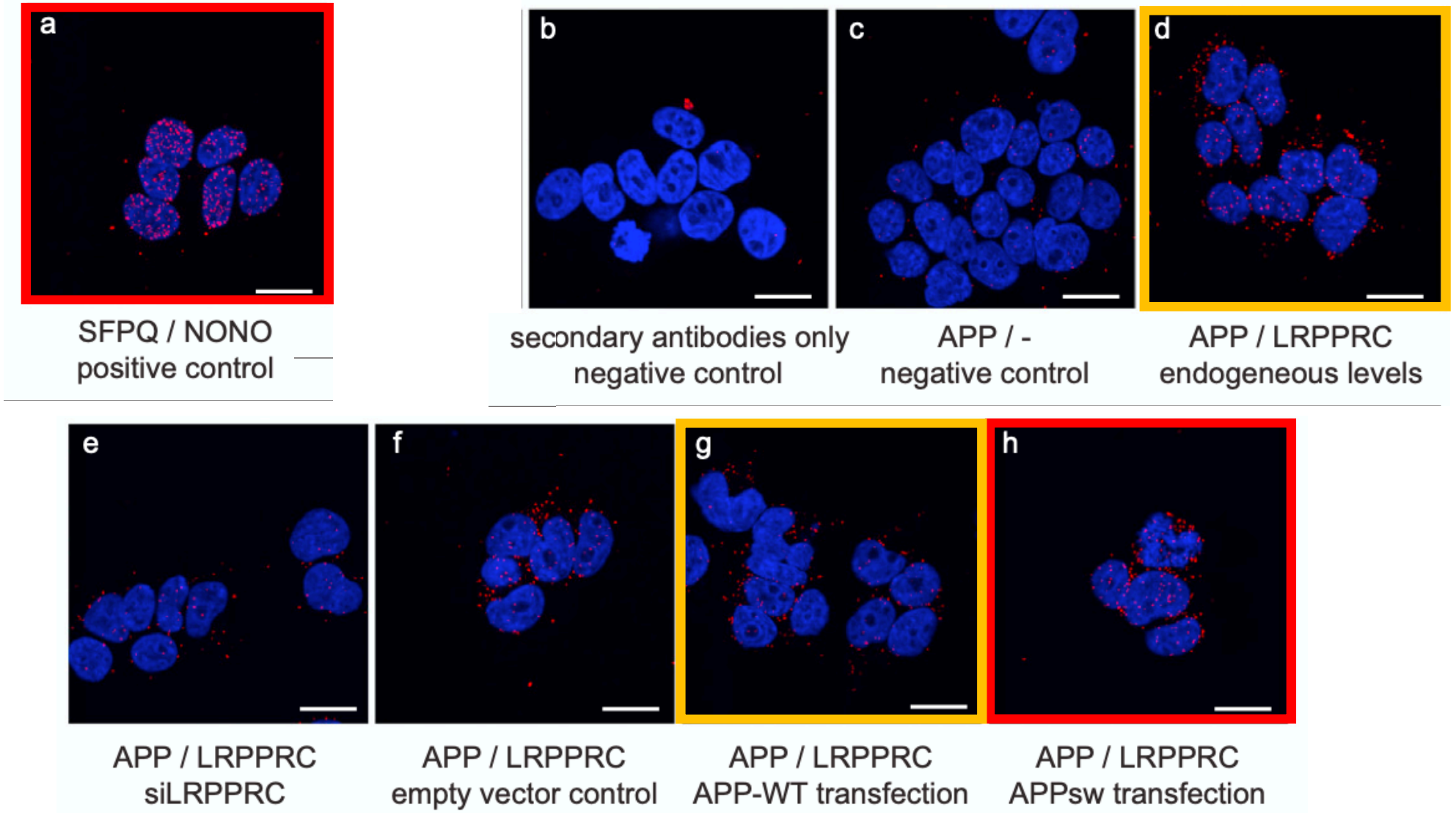
Fig. 5B: Does the "Swedish" variant interact with LRPPRC compared to wildtype?



LRPPRC/APP interaction is affected by APPsw variant in mitochondria.

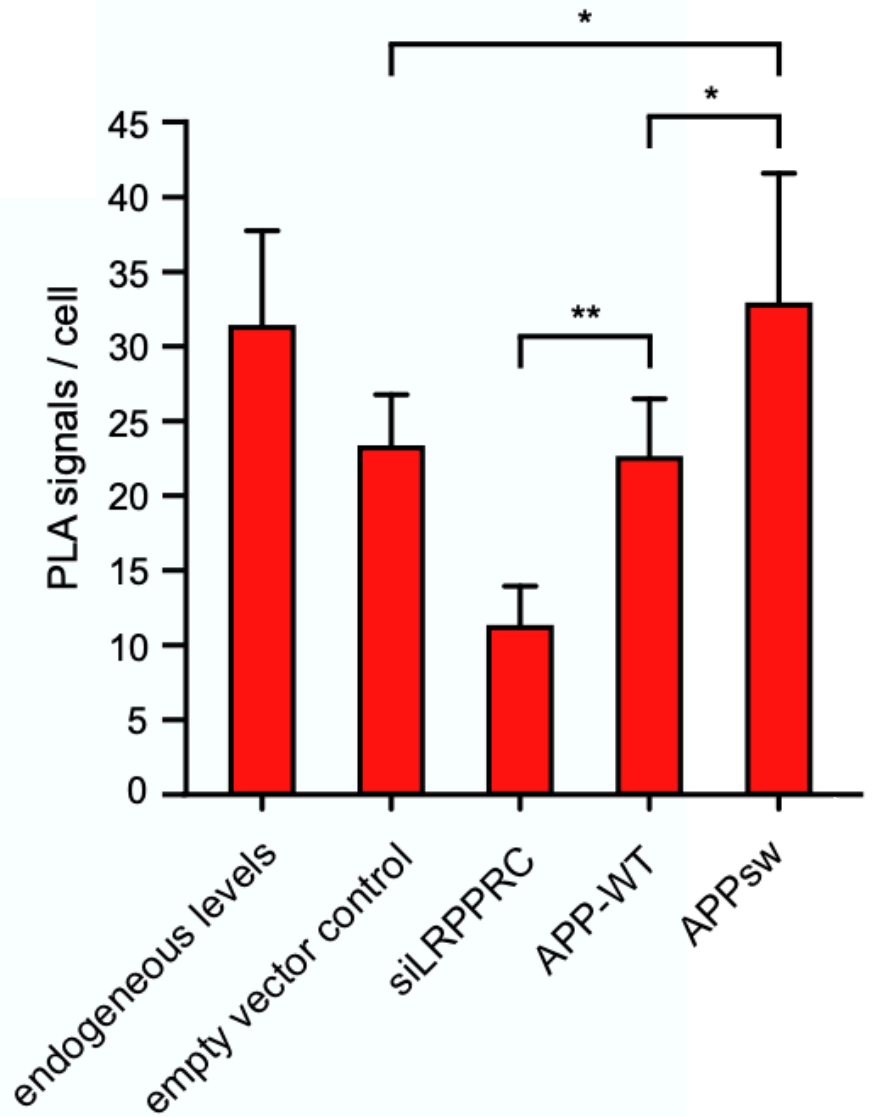
Fig. 5C: How does the "Swedish" variant co-localize with LRPPRC in cells?

APPsw mirrors +control



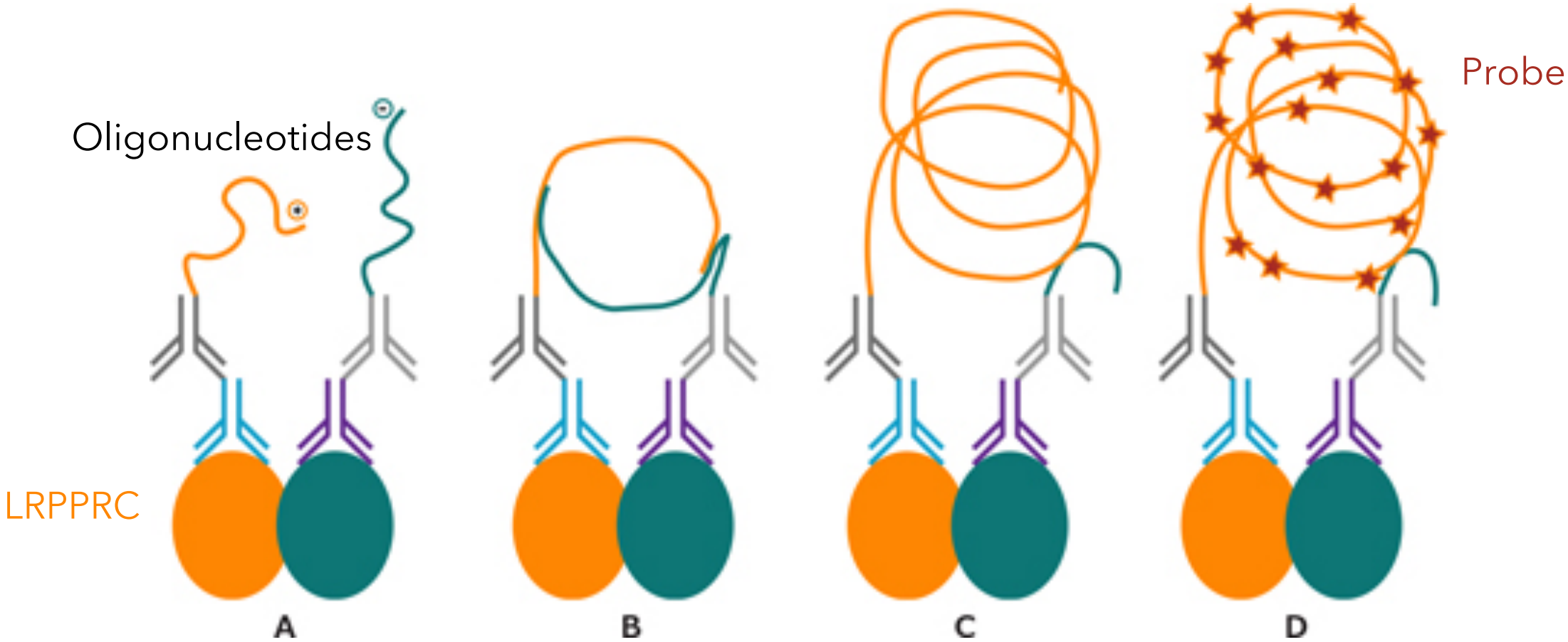
Proximity ligation assay (PLA) in HEK293T cells

Fig. 5D: How was differential binding behavior verified independently from SILAC?



Transfecting *APPsw* increased the signal compared to wild-type

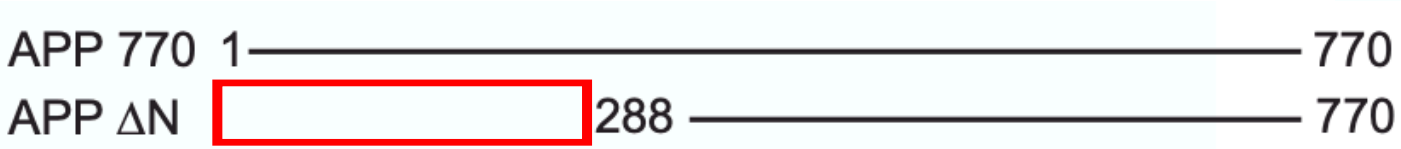
What is a PLA assay?



provides high specificity and sensitivity

Fig. 5F: How do changes in protein binding domains affect interaction?

N-terminus mutations affect APP/LRPPRC interaction



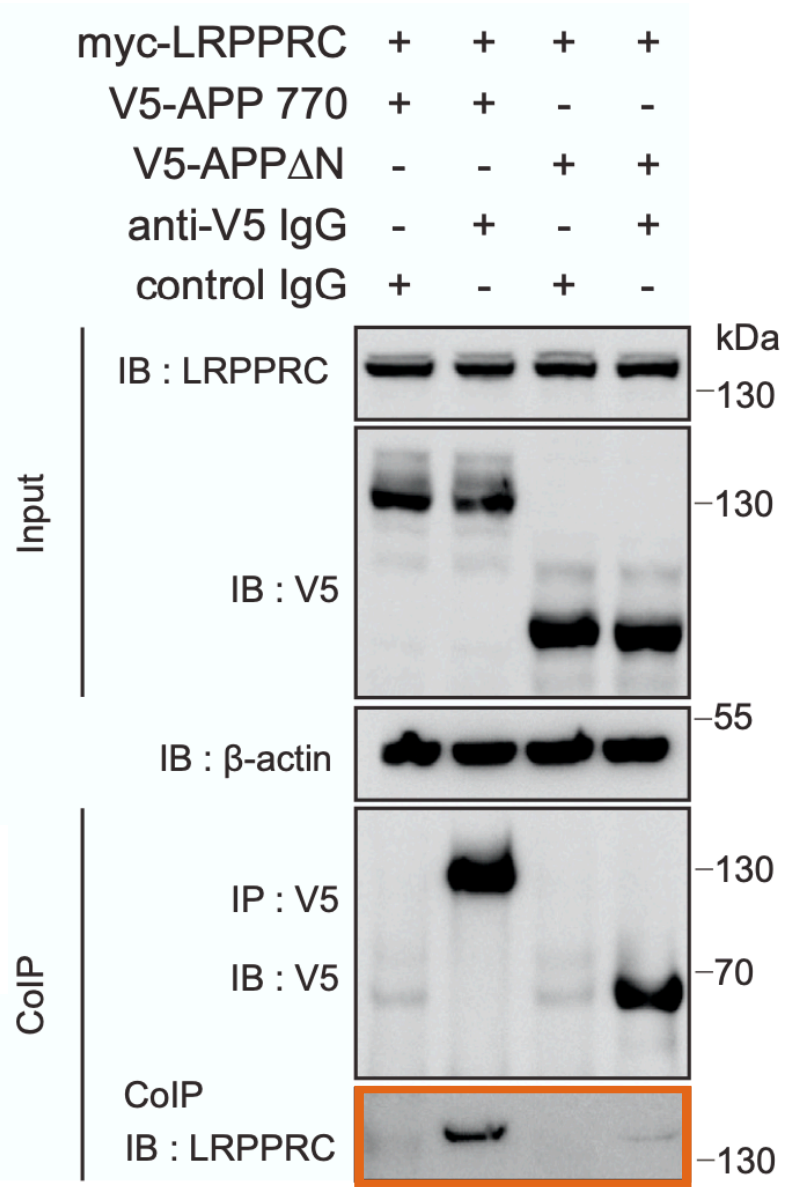


Fig. 5E: How did neurodegenerative-associated protein interactions compare in human brain?

immunohistochemistry reveals **APP** and **LRPPRC** are co-expressed in healthy cells

LRPPRC is not found in amyloid plaques

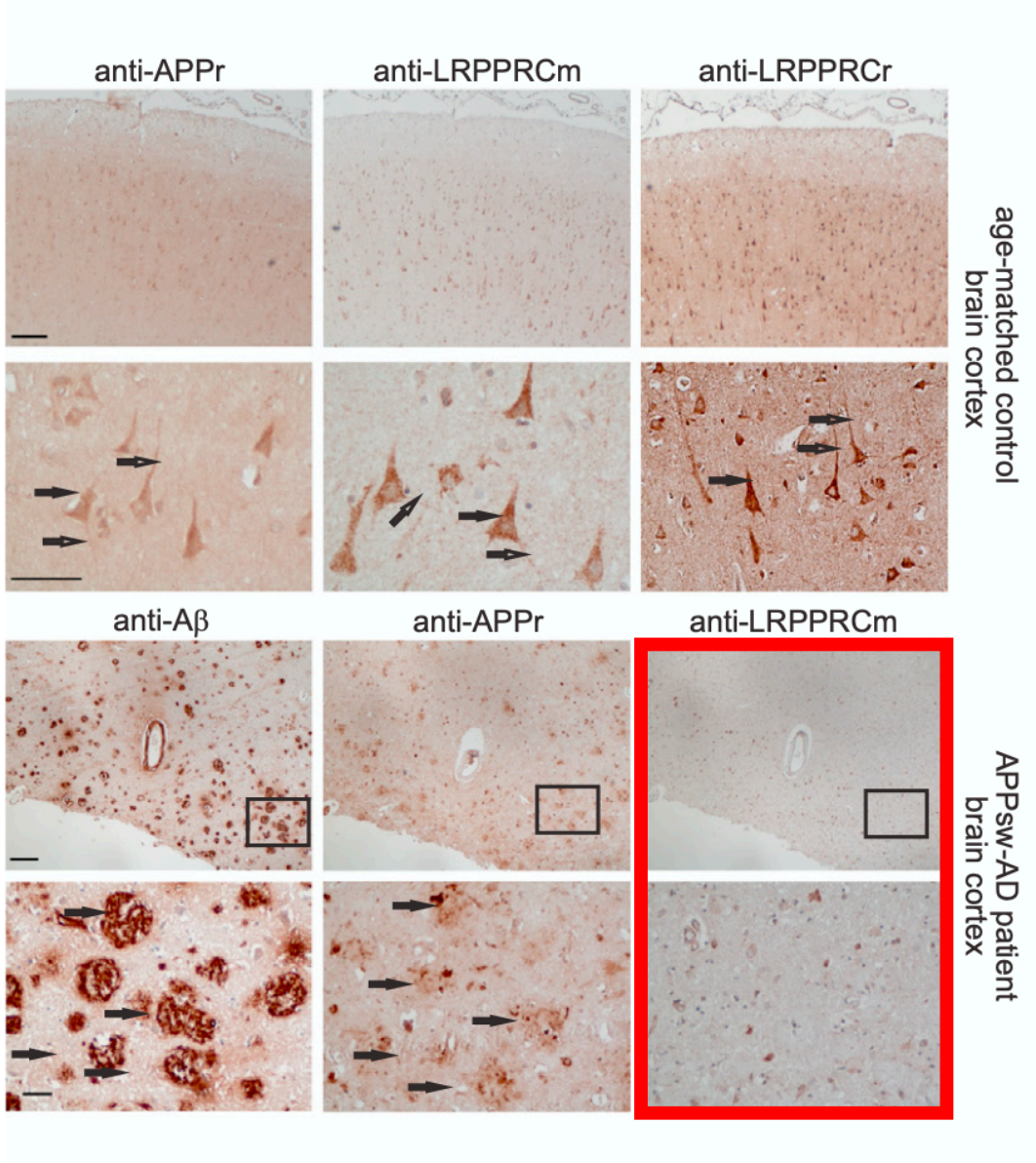


Fig. 6A: How does "Swedish" mutation effect the proteome?

APPsw downregulated cellular levels of LRPPRC and SLIRP

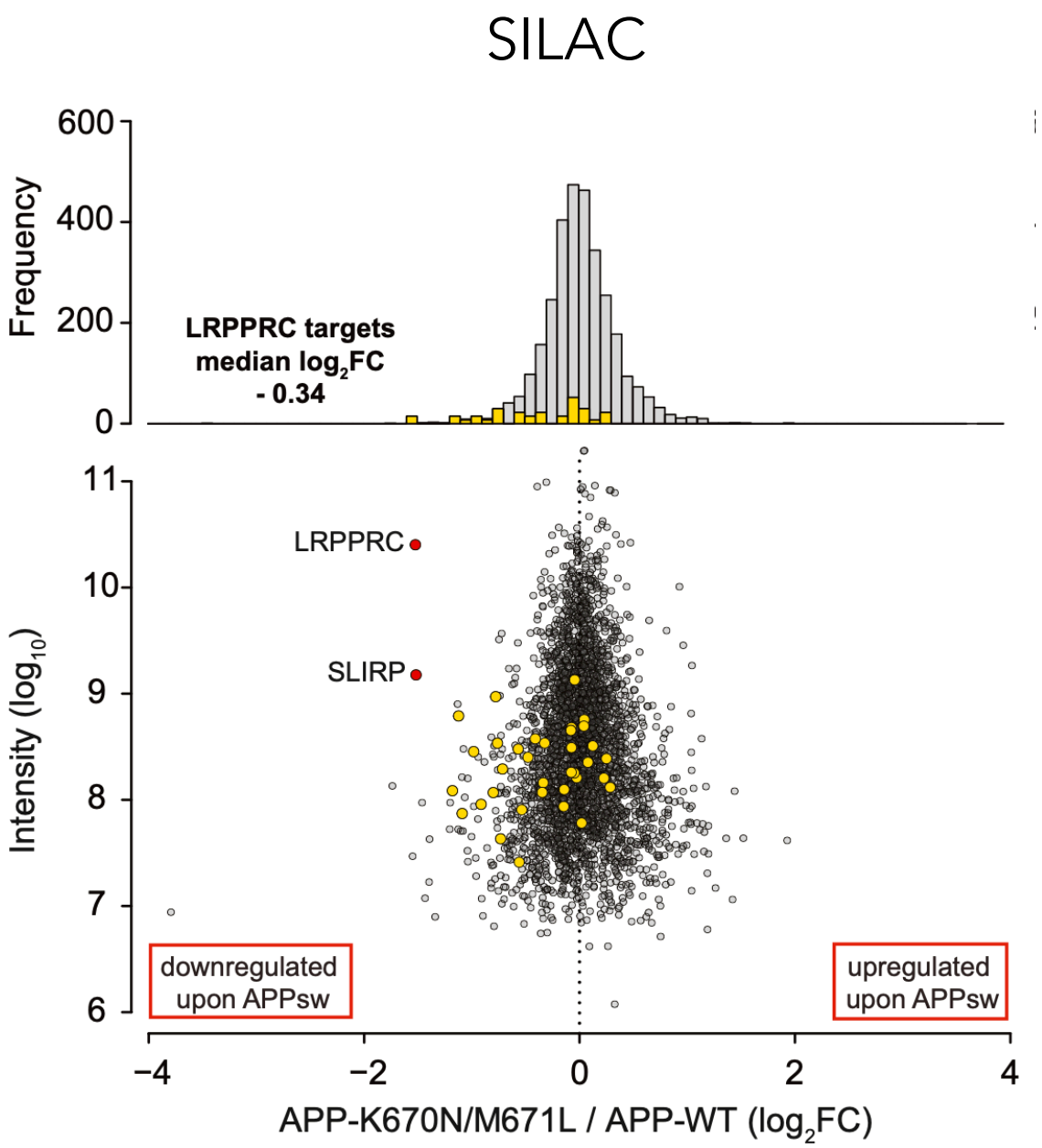


Fig. 6B: How does Co-expression of LRPPRC effect these results?

LRPPRC and SLIRP co-overexpression rescues mitochondrial function

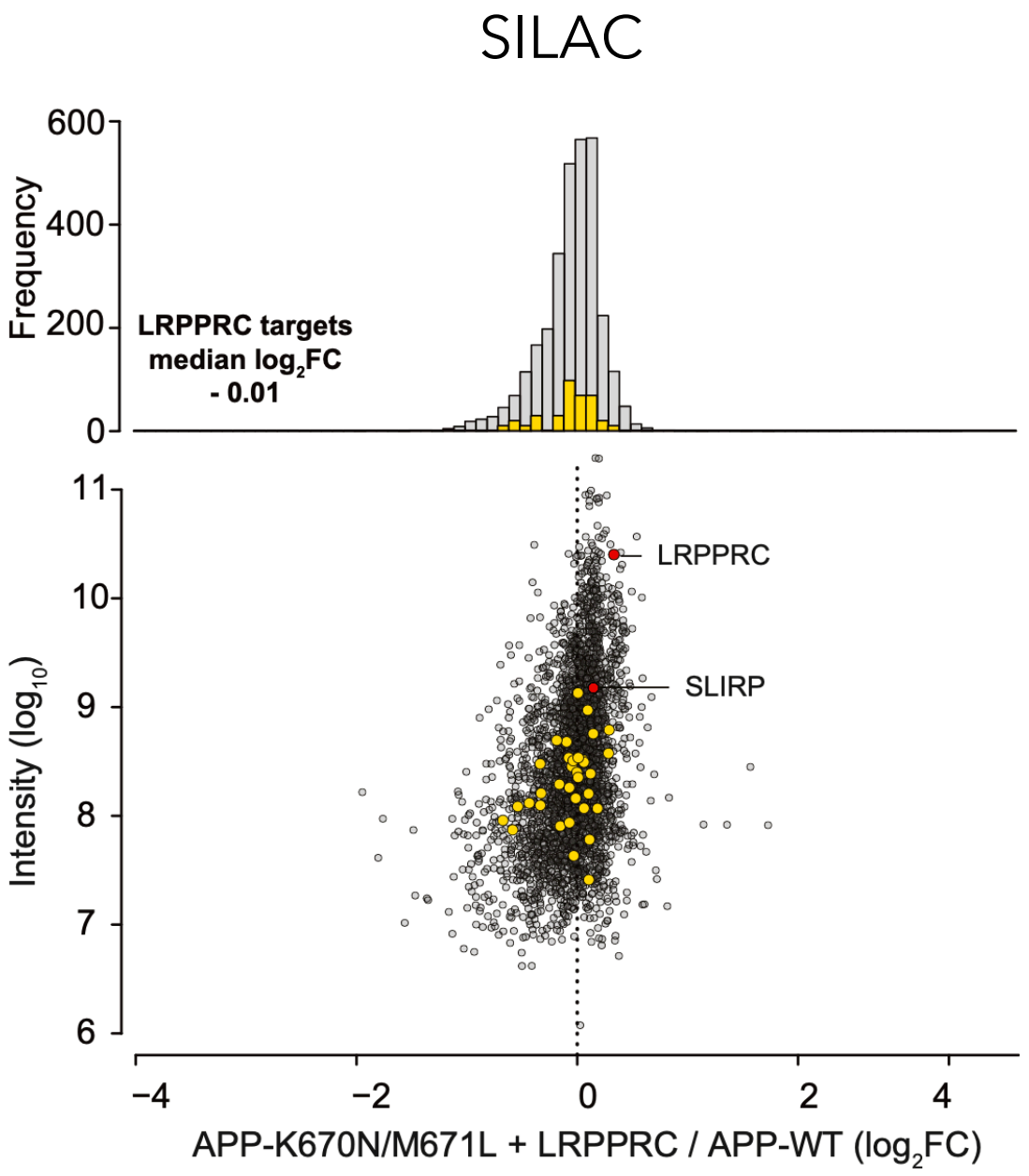
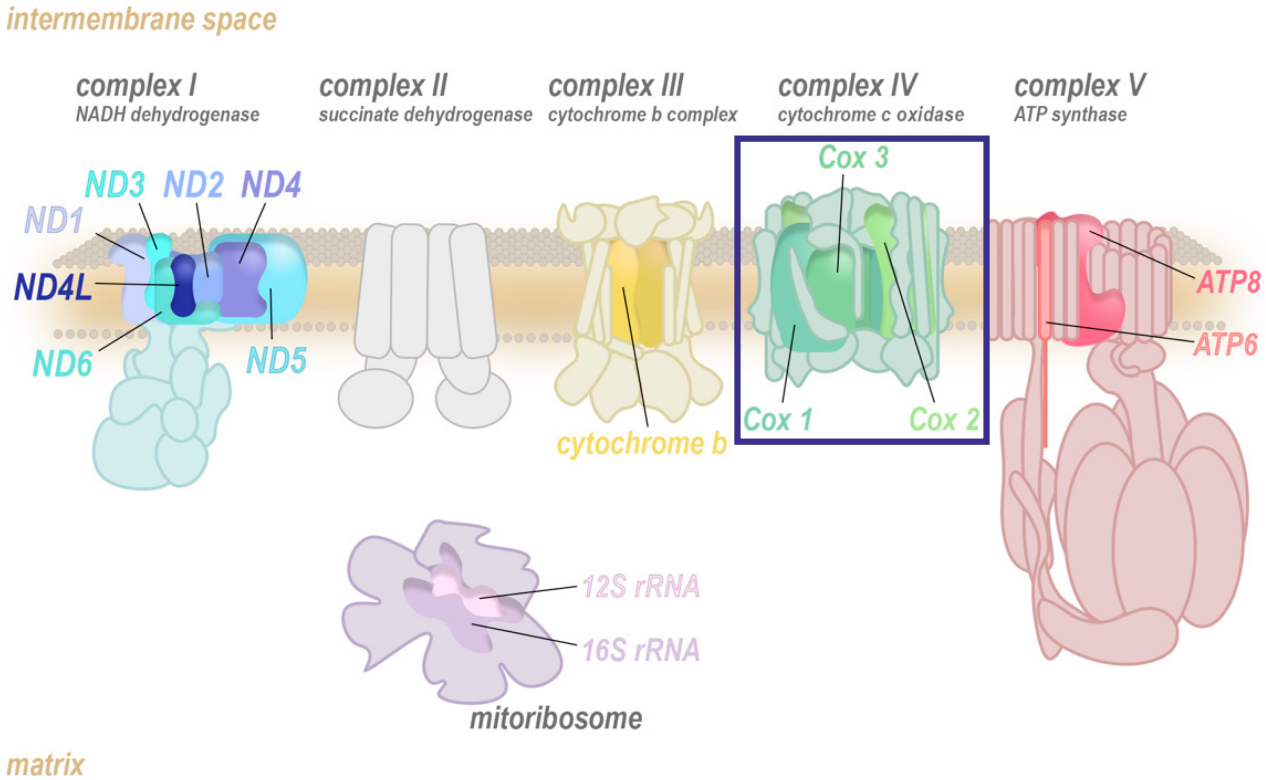


Fig. 6C: How might “Swedish” variants effect the electron transport chain?

APPsw downregulates expression of COX1 & LRPPRC



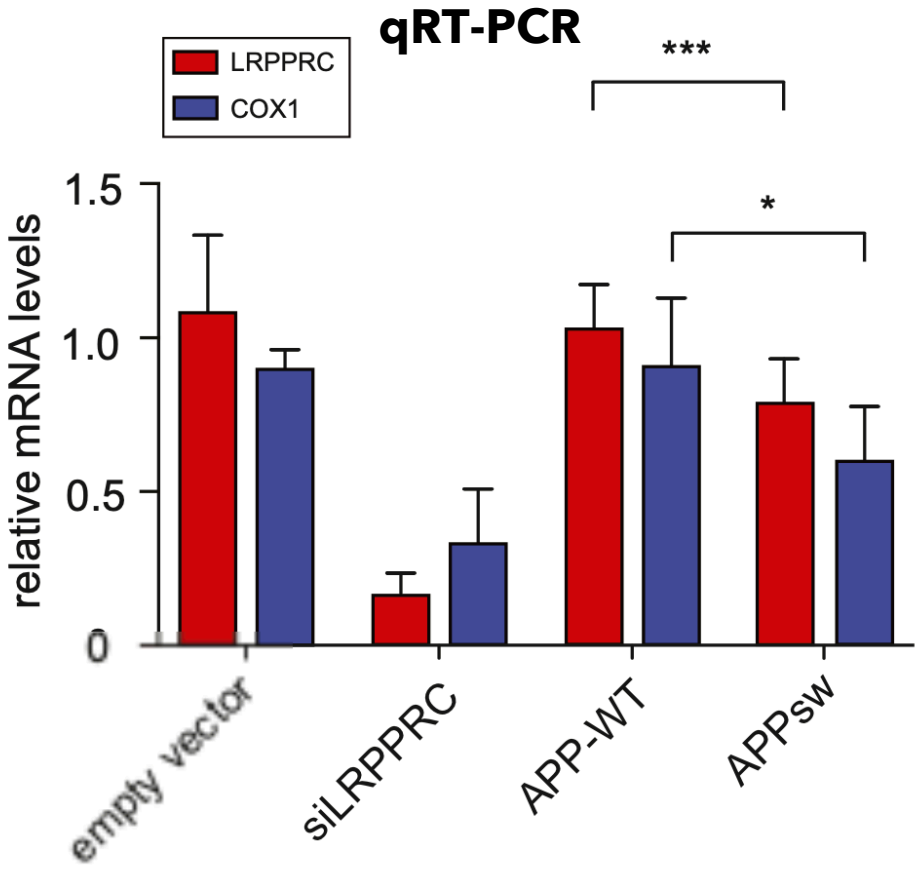
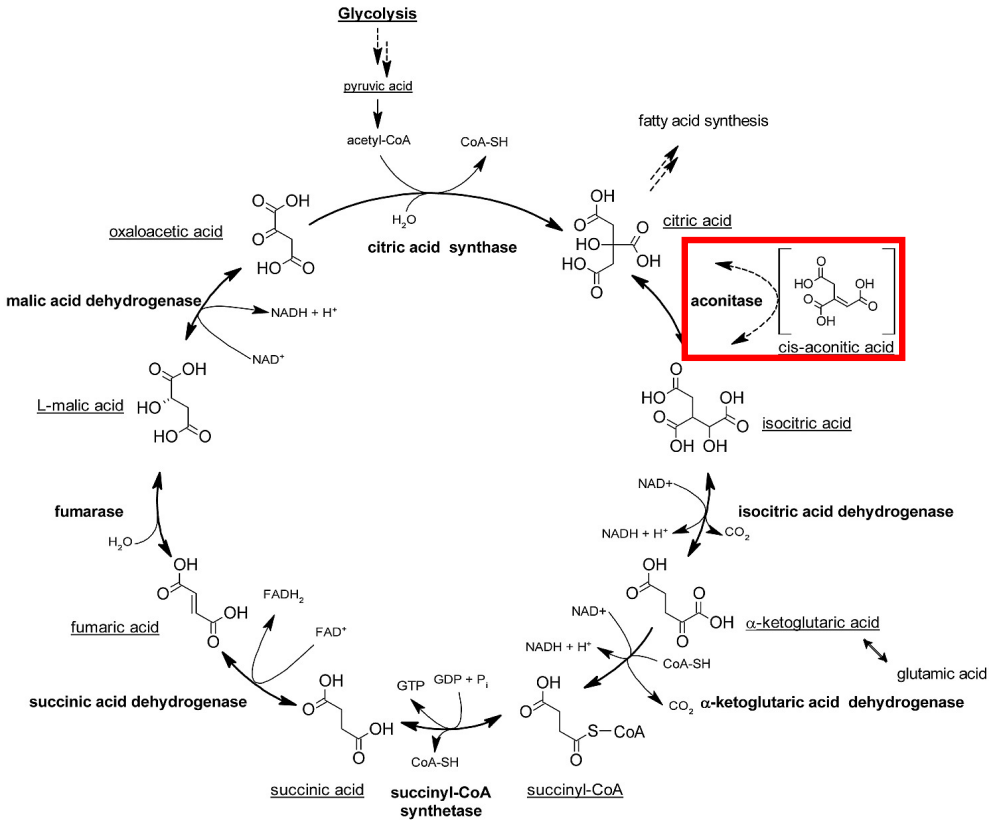


Fig. 6D: How might "Swedish" variants effect oxidative stress?

Aconitase activity represents *oxidative stress* on mitochondria



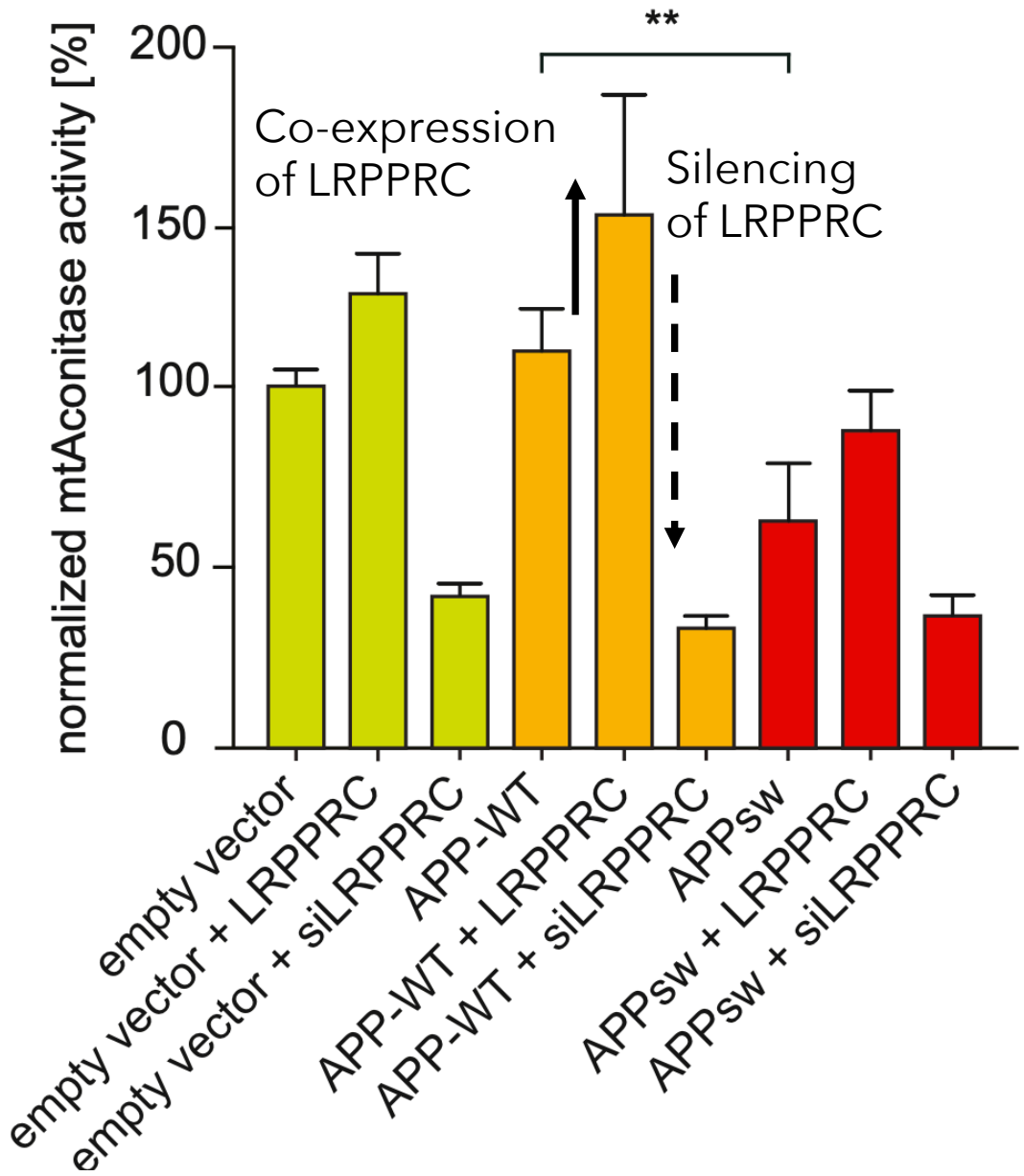
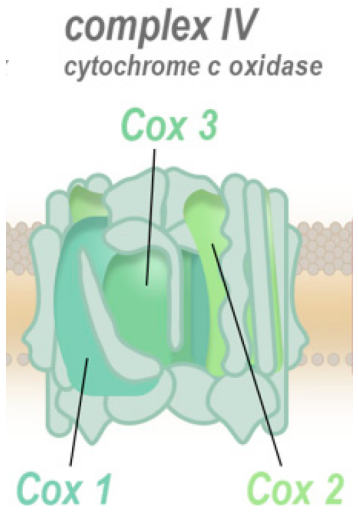
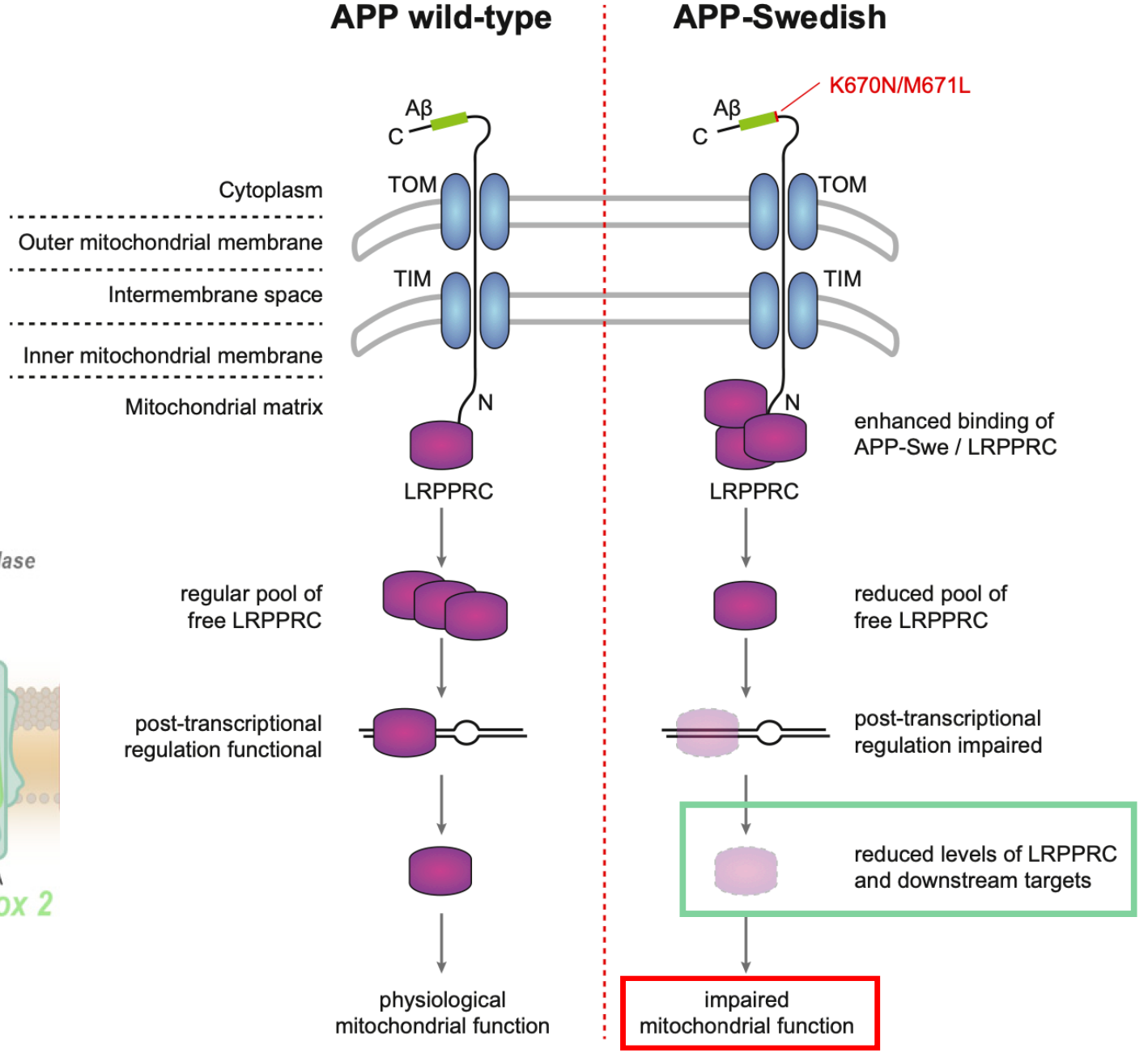


Fig. 7: In summary, how does the “Swedish" variant promote neurodegeneration?

Oxidative stress
&
impaired electron transport chain





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