Lysosomal Dysfunction and Neurodegenerative Diseases: Focusing on the Roles of Zinc and cAMP

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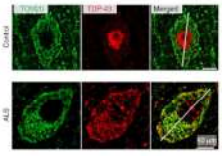
Common key pathology of neurodegenerative diseases

- **Accumulation of protein aggregates**

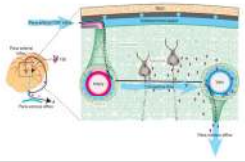
AD: Aβ, p-tau
PD: DLBD: α-synuclein
FTD: p-tau, TDP-43
ALS: TDP-43, SOD1, p-tau
HD: huntingtin
CJD: prion



Is there a common denominator mechanism for these diseases?

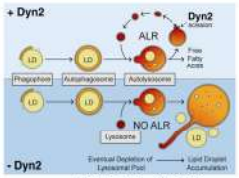
Balance between generation and clearance of protein aggregates

- Generation mechanism may be particular to each disease
- Defects in clearance may be shared by these diseases
  - Autophagy/Lysosomes
  - Exosomes: endosome/lysosome-related vesicles, may be important in extracellular accumulation and transcellular propagation
  - Extracellular degradation (IDE, neprilysin, etc)
  - Bulk clearance of extracellular wastes from the brain: glymphatic system (AQ4-dependent): diurnal variation → possible relevancy to sleep-dependence of Aβ accumulation? (Roh et al., Sci Transl med, 2012; Rainey-Smith et al., Tran L| Psychiatry, 2018))



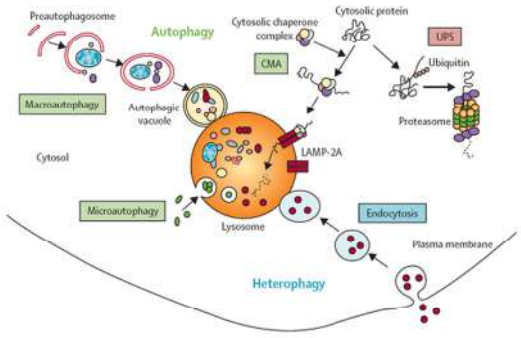
Lysosomes

- Discovered by Christian de Duve (Nobel prize 1974)
- The bulk clearance system in a cell
- Spherical vesicles (0.2-0.8 μm) containing acid hydrolases (>70)
- Enzymes are tagged with mannose-6-phosphate for delivery to lysosomes
- Inside pH, 4.5-5.0 (like stomach): vATPase (proton pump)
- Formation from ER and endosomes and reformation from autolysosomes (ALR)



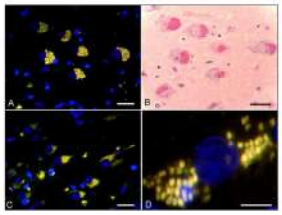
Schulze et al., JCB, 2013)

Lysosome-centered degradation of proteins and organelles: autophagy (self-eating) and endocytosis

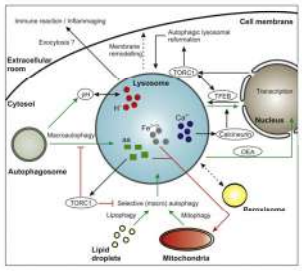


Aging and lysosomal dysfunction

1) aging causes lysosomal dysfunction
2) Longevity-extending conditions converge on lysosomes



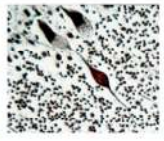
Lipofuscin accumulation in aged human brain
Gray and Woulfe, 2005



Carmona-Gutierrez et al., Ageing Res Rev, 2016

Lysosomal dysfunction causes neurodegeneration:
lysosomal storage disorders (LSD) including neuronal ceroid lipofuscinosis

LSD	DEFECTIVE ENZYME	NEUROLOGICAL FEATURES
SPHINGOLIPIDOSES • GM1 and GM2 gangliosidosis • Niemann-Pick disease (NPC) • Gaucher disease • Others	Lysosomal hydrolases [i.e. *Hexosaminase A* in GM2; *Sphingomyelinase* in NPC; *Glucocerebrosidase* in Gaucher disease]	Progressive neurological regression, seizures, spasticity,
MUCOPOLYSACCHARIDOSES • MPS-III • Others	Glycosaminoglycan cleaving enzymes	Mental retardation, behavioural disturbances and hyperactivity
GLYCOPROTEINOSES • Mucolipidosis • Others	Glycoprotein cleaving enzymes [*N-acetylglucosamine-1-phosphotransferase* in Mucolipidosis I]	Mental impairment, speech impairment, spasticity, neuroaxonal dystrophy
NEURONAL CEROID LIPOFUSCINOSIS • Batten disease • Others	Lysosomal proteins (e.g. proteases) [i.e. *CLN3 in Batten*]	Visual failure, epilepsy, decline in motor and cognitive skills
MULTIPLE SULFATASE DEFICIENCY	Sulfatase modifier	Rapid neurological deterioration

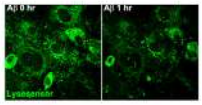


Some lysosome-related genes/proteins involved in other neurodegenerative diseases

- Deletion of the **progranulin** gene in patients with frontotemporal lobar degeneration or Parkinson disease (Rovelet-Lecrux et al., Neurobiol Dis, 2008)
- **Cathepsin D** gene and the risk of Alzheimer's disease: a population-based study and meta-analysis (Schurr et al., Neurobiol Aging, 2011)
- Excessive burden of **lysosomal storage disorder gene variants** in Parkinson's disease (Robak et al., Brain, 2017): CtsD, GBA1 etc.
- Mutated **cathepsin F** in adult-onset neuronal ceroid lipofuscinosis and FTD (der Zee et al., Neurol Genet, 2016)
- **CLN3** protein regulates lysosomal pH and alters intracellular processing of Alzheimer's amyloid-beta protein precursor and cathepsin D in human cells (Mol Genet Metab, 2000)
- ----------

Some pathologic proteins of neurodegenerative diseases and lysosomal functions

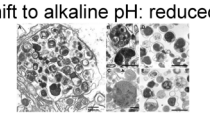
- Aβ inhibits lysosomal function: a vicious cycle?
- TMEM106B (FTD risk factor): impairs lysosomal acidification (Busch et al., Hum Mol Genet, 2016)
- TDP-43 (FTD, ALS) loss of function: autophagosome-lysosome fusion defect (Xia et al., EMBO J, 2016)
- Mutant presenilin-1: impairs lysosomal acidification
- α-synyclein inhibits its own degradation (Bourdenx et al., Front Neuroanat, 2014)



How to overcome lysosomal dysfunction?

<u>Two common abnormalities</u>

1. Failed fusion between autophagosome/endosome and lysosome ("arrested autophagy): accumulation of wastes in autophagosomes and endosomes
2. Shift to alkaline pH: reduced degradation

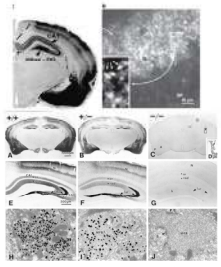


<u>As possible therapeutics, we focused on</u>

1. zinc signaling
2. cAMP (PDE inhibitors)

Zinc in Brain

- **A large amount of zinc in brain**
  - 100–150 μM in grey matter
  - Most zinc is protein-bound
  - 10–20% in synaptic vesicles of glutamatergic terminals (free, labile or loosely-bound): ZnT3-dependent
  - After intensive synaptic activity, extracellular zinc concentrations may reach μM levels
  - Zinc transporters: ZIPs and ZnTs
  - Zinc influx: ZIPs, PrPc, CAK channels, VGCC, NMDA channels, Na-K exchangers
  - Intracellular zinc release: zinc proteins such as metallothioneins (Mt3), organelles (ER, lysosomes, mitochondria)



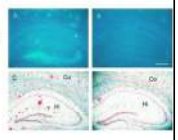
Cole et al., ***PNAS***, 1999

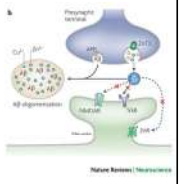
Physiological roles

- Modulation of NMDA-R, GABA-R etc.
- Synaptic plasticity: BDNF/Trk, Src etc.
- Neurogenesis
- Others

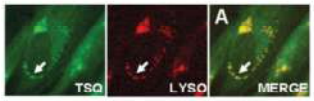
Zinc and Alzheimer's disease (AD)

- Zinc promotes aggregation of Aβ (Bush et al., Science, 1994)
- Zinc in plaques and tangles of mice and humans (Lee et al., J Neurosci 1999; Suh et al., Brain Res 2000)
- Synaptic zinc and AD (Lee et al., PNAS 2002)
- Zinc chelators reduces Aβ pathologies (Cherny et al., Neuron 2001; Lee et al., Neurobiol Aging 2004)
- Trapping of zinc in plaques may result in zinc stasis and synaptic dysfunction (Sensi et al., Nat Rev Neurosci, 2009)
- Other effects of zinc in AD or other neurodegeneative diseases?

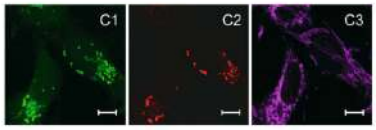




Possible roles of zinc in autophagy/lysosomal degradation? First clue: Free zinc in lysosome (zincosome)

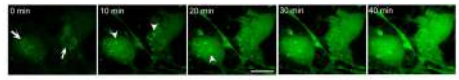


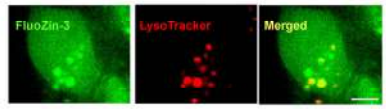
Varea et al. (2006) *Gila* **54**:304-315



Muylle et al. (2006) *BioMetals* **19**:437-450

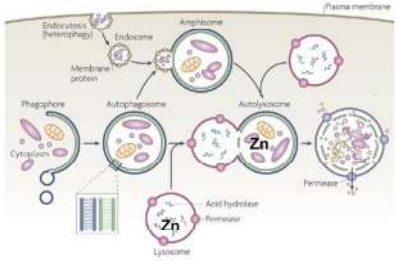
Dynamic changes of free zinc in lysosomes



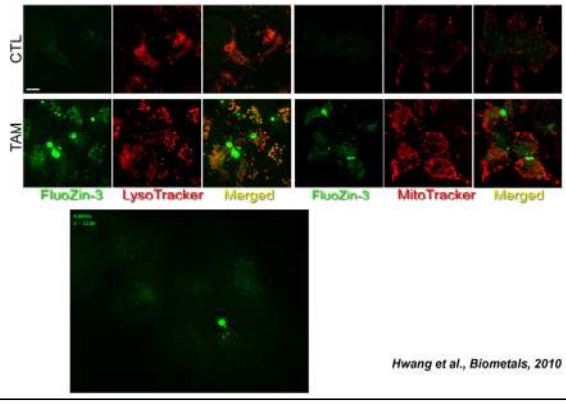


Hwang et al., J Neurosci, 2008

Lysosomal zinc levels change: what is the functional role?

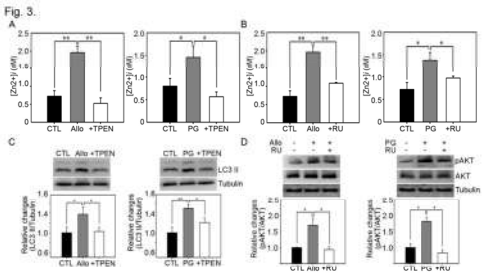


Increases in lysosomal free zinc in autophagy (tamoxifen in MCF7 cells)



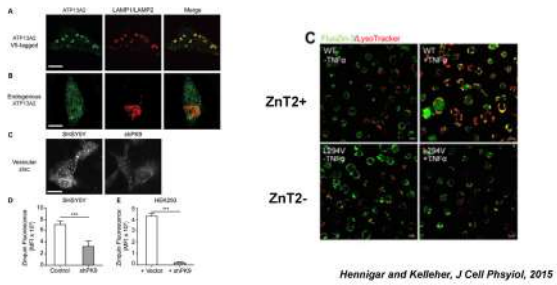
Hwang et al., Biometals, 2010

Essential role for lysosomal zinc in autophagy flux (neurosteroids)



Kim et al., Neurochem Int 2012

Possible zinc transporters for zinc entry into lysosomes: PARK9, ZnT2, ZnT4 and others?



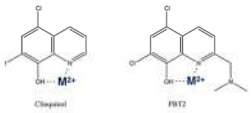
Hennigar and Kelleher, J Cell Physiol, 2015

Kong S M et al. Hum. Mol. Genet. 2014

What may be the physiological significance of free zinc changes in lysosomes?

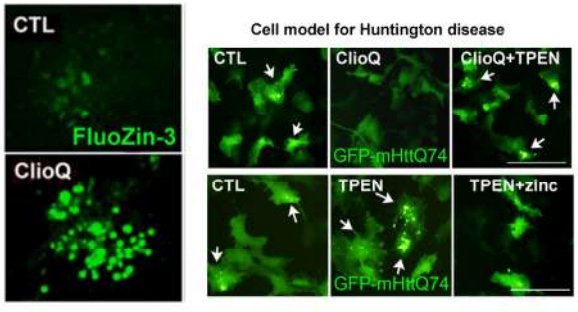
- Does zinc promote lysosomal degradation?
- Would increasing lysosomal zinc with zinc ionophores be an effective measure to promote degradation of abnormal proteins such as Aβ?
- Other measures to enhance lysosomal functions?

Zn-Clioquinol



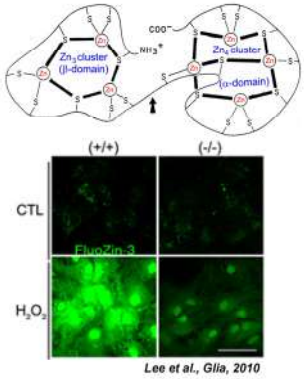
- Zinc chelator with stability constant 8.8 (high nM range)
- Membrane-permeant
- Can function as a zinc ionophore: delivers zinc across membranes
- Used as topical antimicrobial (antibacterial, antifungal)
- Used in Japan as oral anti-amebic drug → subacute myelo-optic neuropathy (SMON)
- Clioquinol-derived PBT2 was tried as anti-AD drug
- Increases zinc in lysosomes

Zn-clioquinol increases lysosomal free zinc and augment the clearance of protein aggregates



Park et al., Neurobiol Dis, 2011

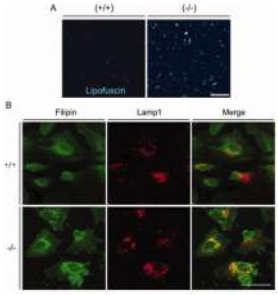
Where does zinc come from? Perhaps metallothionein-3 (Mt3)



Mt3 is mainly expressed in CNS
Mt3 is downregulated in AD brains
(Uchida et al., Neuron, 1991)

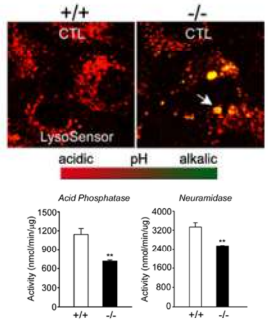
Lee et al., Glia, 2010

The absence of Mt3 causes "arrested autophagy": accumulation of lipofuscin and cholesterol derivatives in lysosomes



Lee et al., Glia, 2010

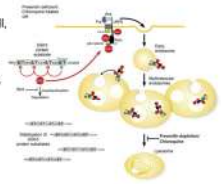
Mt3-null state alters lysosomal pH in astrocytes



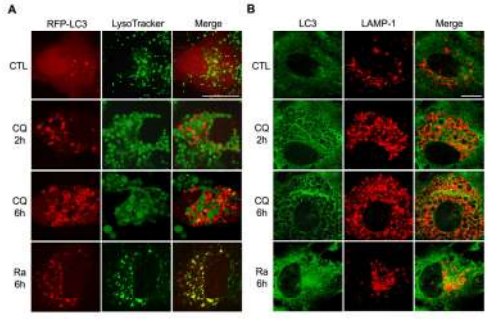
Lee et al., Glia, 2010

The absence of Mt3 or deficiency in lysosomal zinc → Arrested autophagy: a contributing mechanism in aging and neurodegenerative diseases?

- Mt3 is downregulated in AD (Uchida et al., Neuron, 1991)
- Autophagy failure as the key mechanism in AD (Nixon and Yang, Neurobiol Dis 2012)
- Presenilin-1 regulates v-ATPase delivery to lysosomes. PS-1 mutation results in lysosomal alkalinization and arrested autophagy (Lee et al., Cell, 2010)
- Autophagy failure in Alzheimer's disease and the role of defective lysosomal acidification (Wolfe et al., Eur J Neurosci, 2013)

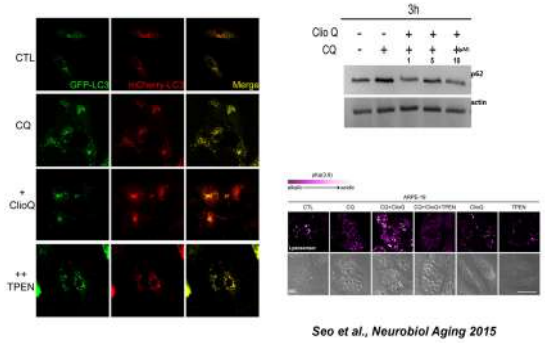


A cell model for arrested autophagy: Chloroquine



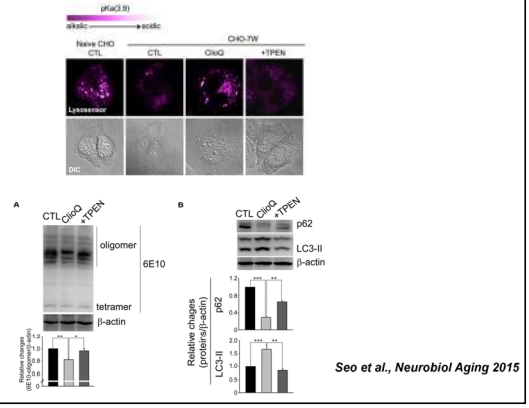
Yoon et al., IOVS, 2010

Delivering zinc to lysosomes (Zn-Clioquinol) can overcome choloroquine-induced arrested autophagy



Seo et al., Neurobiol Aging 2015

mPS1-mAPP expressing cells: Zn-clioquinol reacidifies lysosomes and reduces accumulation of Aβ



Seo et al., Neurobiol Aging 2015

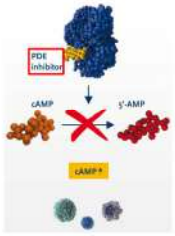
cAMP, autophagy, and lysosomal pH

cAMP induces autophagy via a novel pathway involving ERK, cyclin E and Beclin 1

LYSOSOMAL ALKALIZATION AND DYSFUNCTION IN HUMAN FIBROBLASTS WITH THE ALZHEIMER'S DISEASE-LINKED PRESENILIN 1 A246E MUTATION CAN BE REVERSED WITH CAMP

PDE inhibitors and autophagy

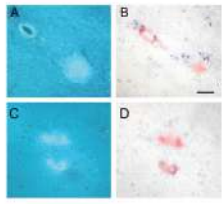
PDE inhibitors



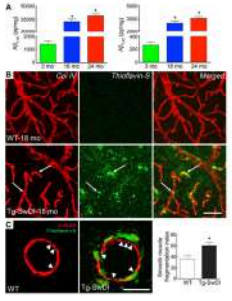
inhibitors	company	indication	BBB permeability	target
Vinpocetine	Richter Gedeon Nyrt.	vasodilation	Yes	PDE1
BCA909	BioCrea	N/A	Yes	PDE2
Cilostazol	Otsuka Pharmaceutical	antiplatelet	Yes	PDE3
Ibudilast	MediciNova Inc.	vasodilation	Yes	PDE4
Sildenafil	Pfizer	vasodilation	Yes	PDE5
Zaprinast	N/A	N/A	N/A	PDE6
BRL-50481	GlaxoSmithKline	N/A	N/A	PDE7
Dipyridamole	Lannett Company Inc.	antiplatelet	No	PDE8
N/A	N/A	N/A	N/A	PDE9
Papaverine	American Regent Inc	vasodilation	N/A	PDE10

BOTH 1 2 3 10 11
cAMP 4 7 8
cGMP 5 6 9

Cerebral amyloid angiopathy, zinc, and pericytes

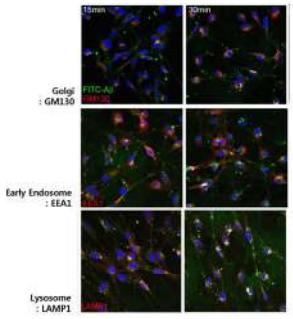


Friedlich et al., J Neurosci, 2004

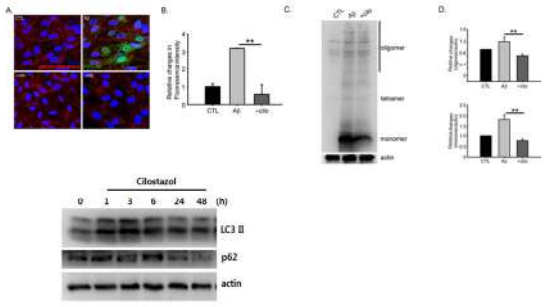


Park L et al. Stroke. 2014

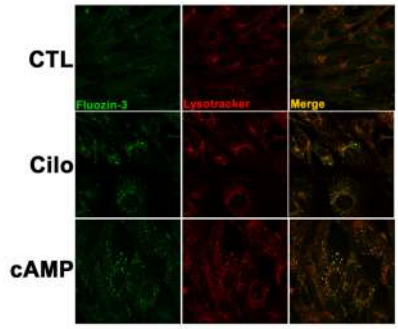
Aβ uptake and its accumulation in endosomes and lysosomes of pericytes



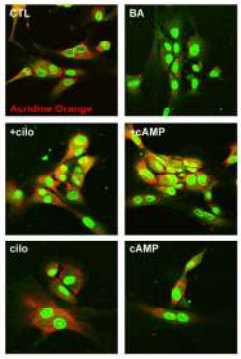
Cilostazol (PDE3 inhibitor) activates autophagy and reduces Aβ burden in pericytes



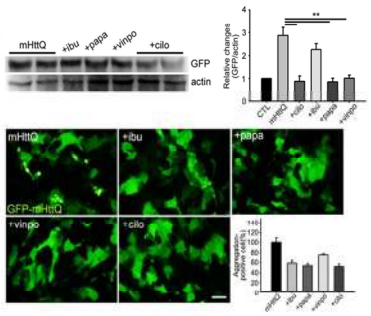
Cilostazol and cAMP increase zinc in lysosomes



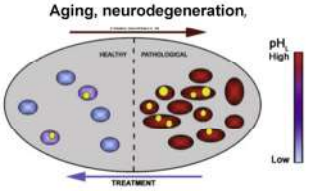
Cilostazol and cAMP restore lysosomal acidity



PDE inhibitors reduce huntingtin aggregates in astrocytes



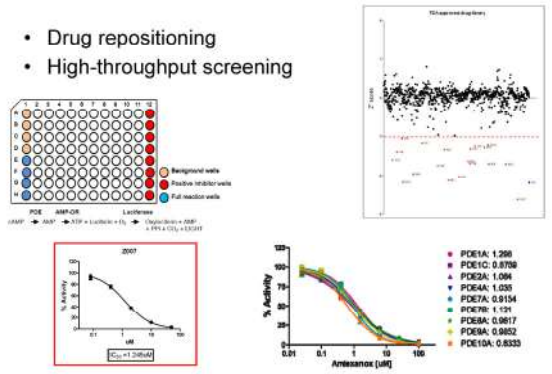
Zinc and cAMP (PDE inhibition) may acidify lysosomes and boost lysosomal degradation



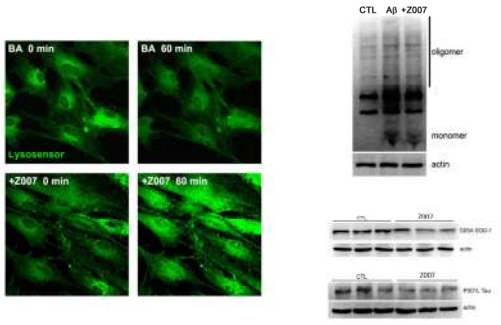
Zn, cAMP

A strategy to find new PDEi

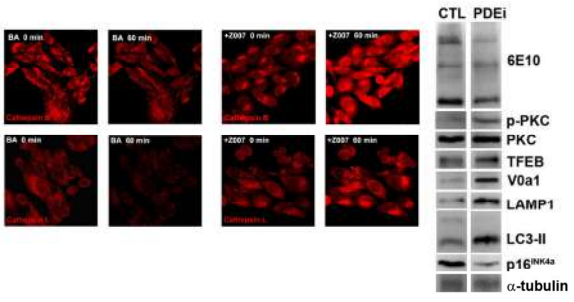
- Drug repositioning
- High-throughput screening



New PDEi (Z007) restores lysosomal acidity and reduces protein aggregates



Z007 restores cathepsin B and D activities



Conclusion and questions

- Zn and MT3 positively regulate lysosomal functions
- Increases in intracellular (lysosomal) free zinc (e.g. clioquinol) may help enhance autophagic degradation
- PDE inhibition (increases in cAMP) may facilitate lysosomal degradation through lysosomal acidification, partly by increasing free zinc

- ***Therapeutic potentials in aging and neurodegenerative diseases?***