Drug Transporter Studies:
Lysosomal Trapping

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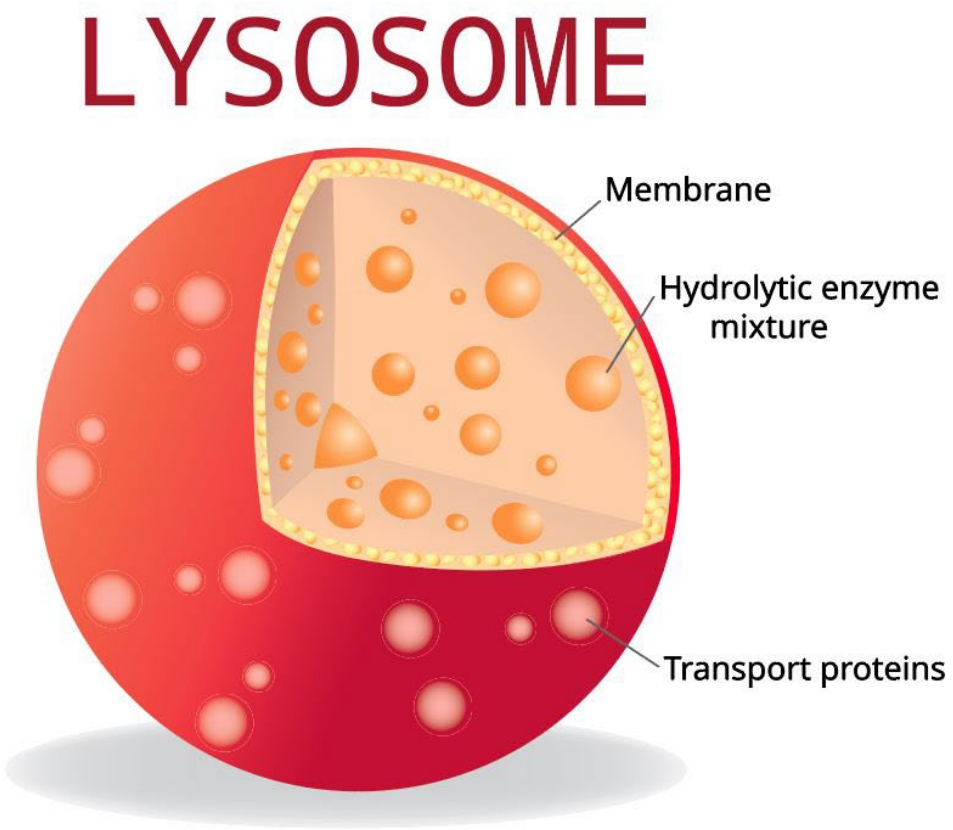
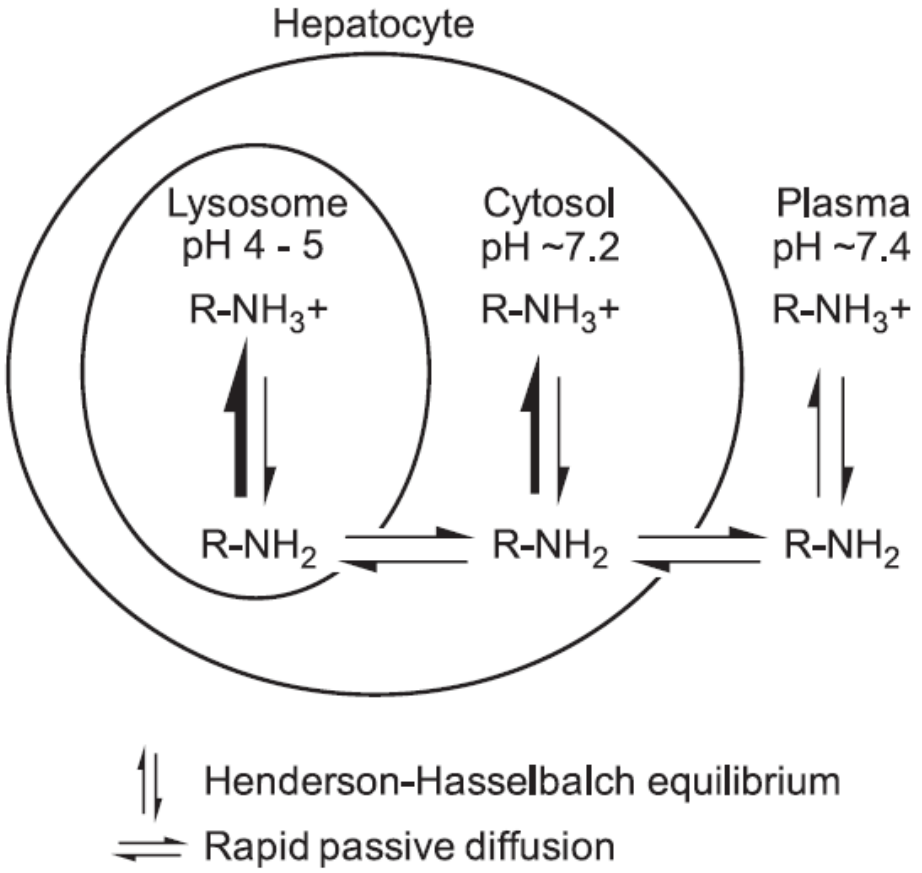
What are Lysosomes?

- Resident macrophage organelle in eukaryotic cells
- Acidic environment (pH ~4-5)
- Hydrolytic enzymes
- Break down larger molecules (e.g., proteins, polysaccharides, lipophilic compounds)
- ~1% of hepatic cell volume



Mechanism of Lysosomal Trapping

- Lysosomotropism (lysosomal trapping) is a physicochemical process
- Cationic amphiphilic compounds (many CNS and cardiovascular drugs)
- logP > 1, pK_a > 6.5
- Diffuse easily across membranes
- Become protonated (+ charge) in lysosome and become trapped



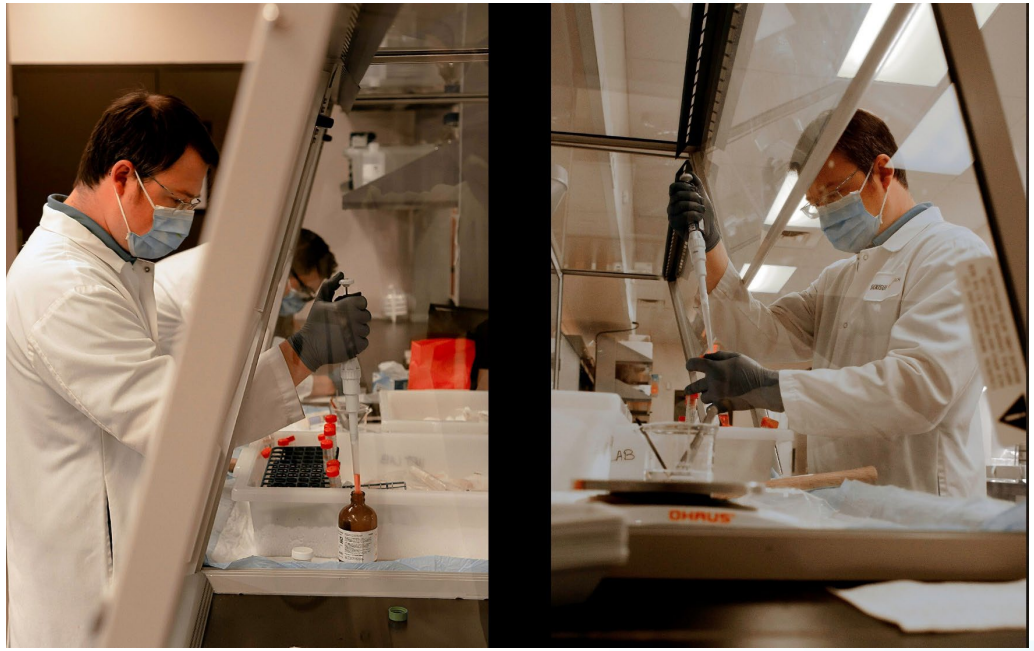
From Kazmi et al., 2013, DMD

Lysosomal Trapping and DDI

- Can lead to high organ-to-blood ratios, often mistaken for active drug transport
- Competition for lysosomal trapping, concomitant administration of lysosomotrpics could lead to elevated drug exposure levels
- Accumulation of lipophilic amines can lead to drug-induced phospholipidosis due to decreased phospholipid catabolism

Lysosomal Trapping Study Designs

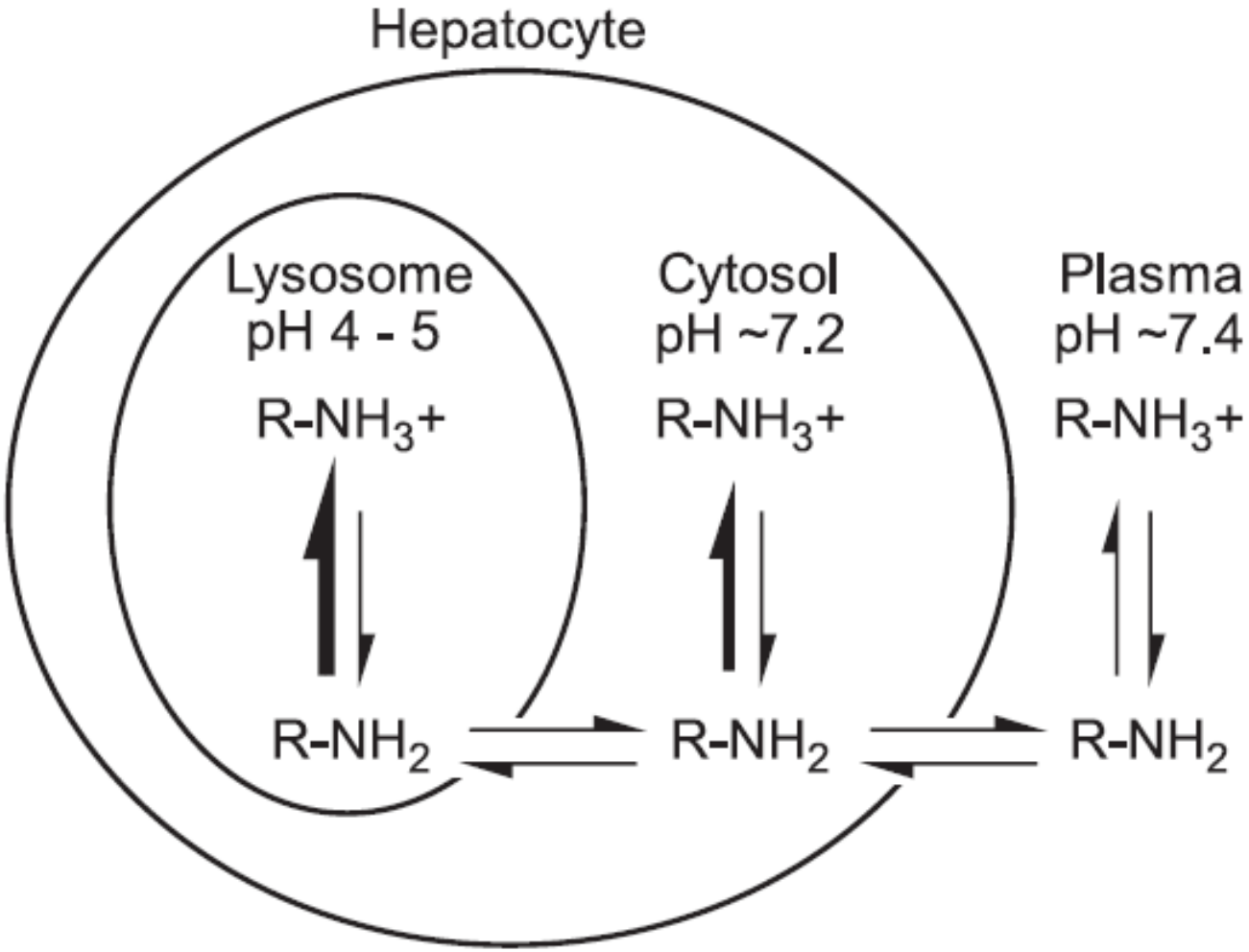
Studies are conducted in Fa2N-4 cells (immortalized hepatocytes) that don't have a lot of drug metabolizing enzymes



1) LysoTracker Inhibition Assay (screen)
   - Lysotracker Red used as a fluorescent probe (measured by fluorescence)
   - Six test article concentrations
2) Mechanistic Determination (definitive)
   - TA measured by LC/MS
   - 2 [TA], 2 time points, w/ and w/o NH_4Cl
   - Positive control propranolol w/ and w/o NH_4Cl
   - If worries of ammonium chloride interacting with TA, chloroquine can be used instead

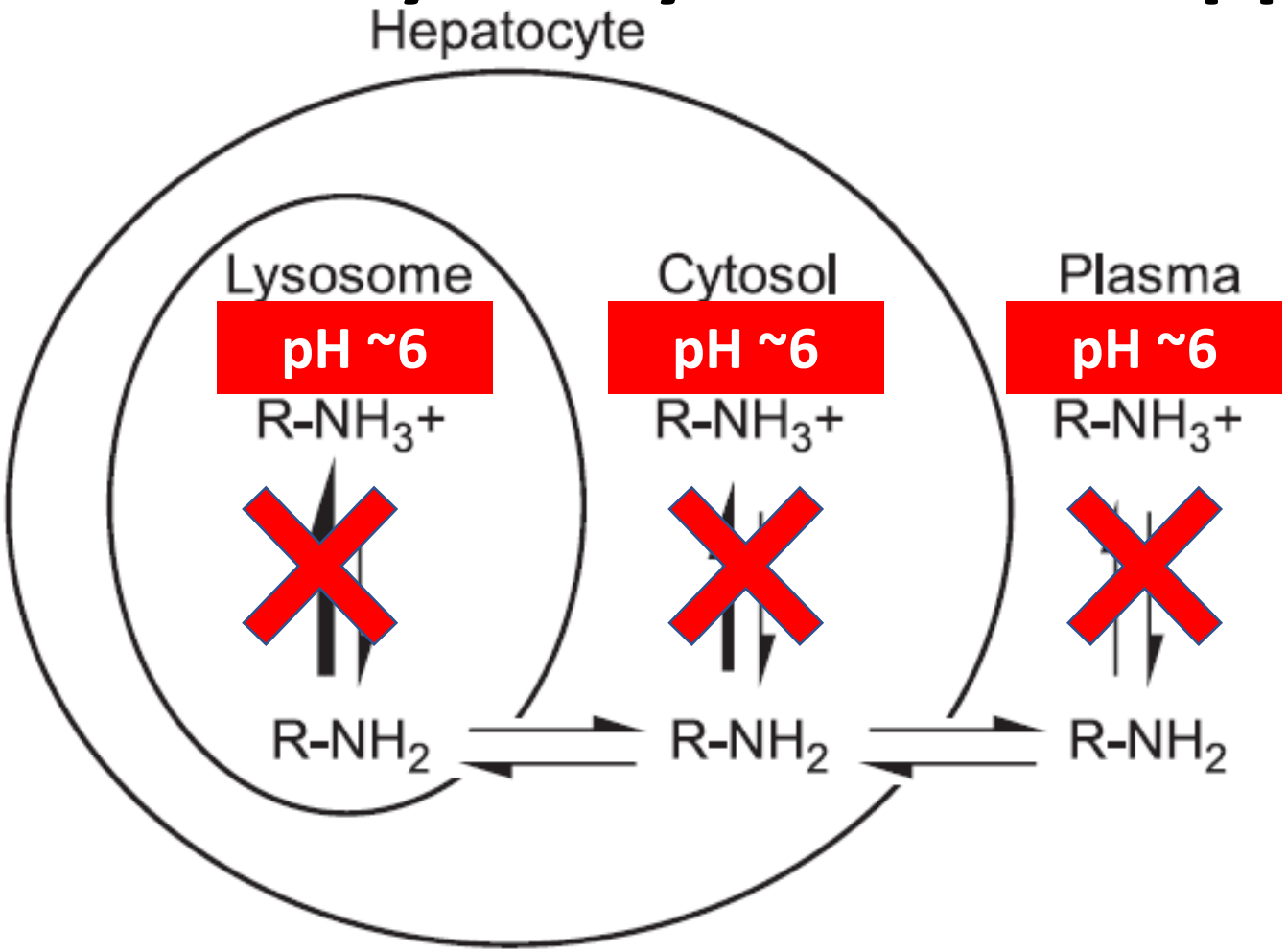
Mechanistic Assay for Lysosomal Trapping

Without NH_4Cl



Mechanistic Assay for Lysosomal Trapping

With NH_4Cl



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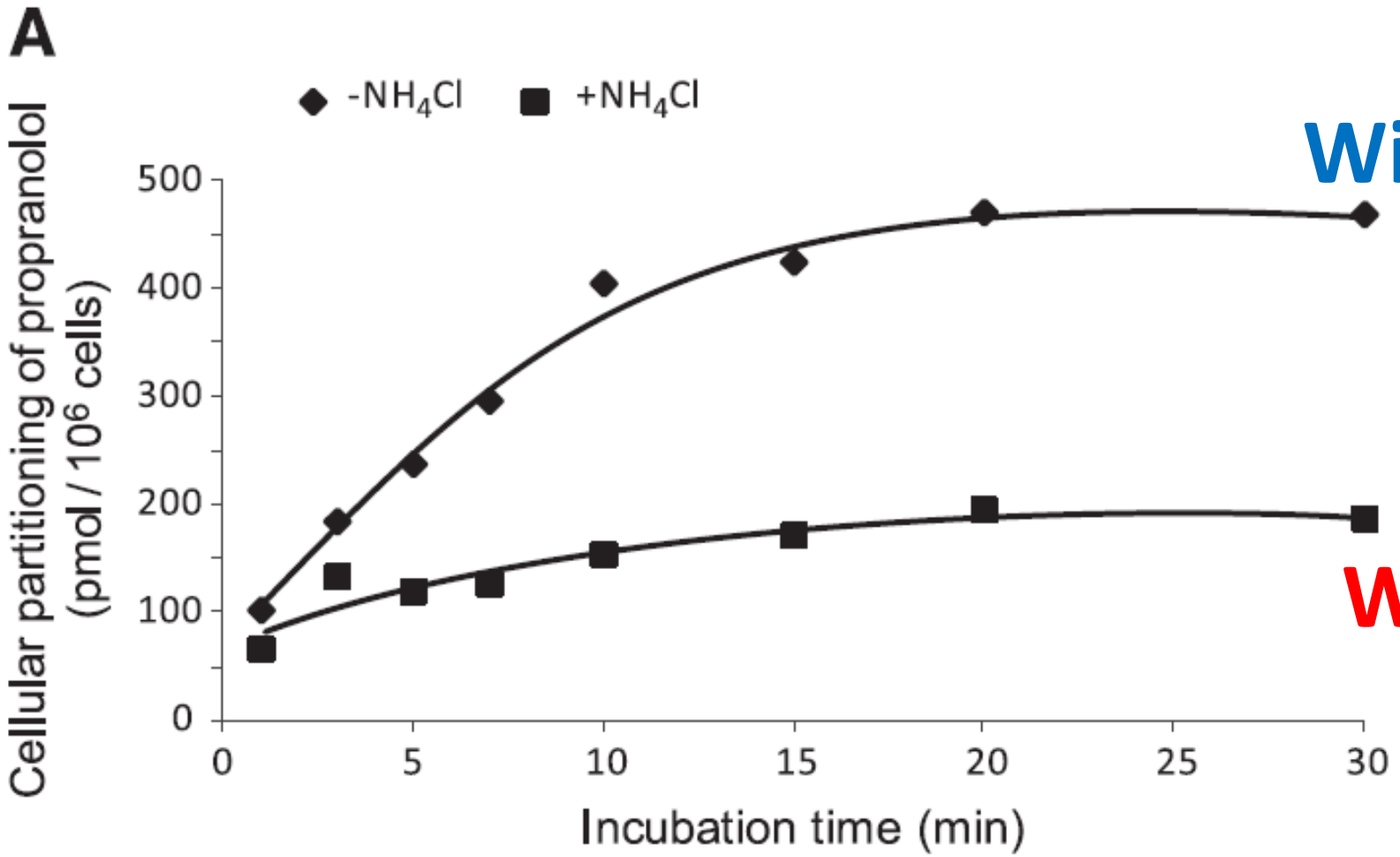

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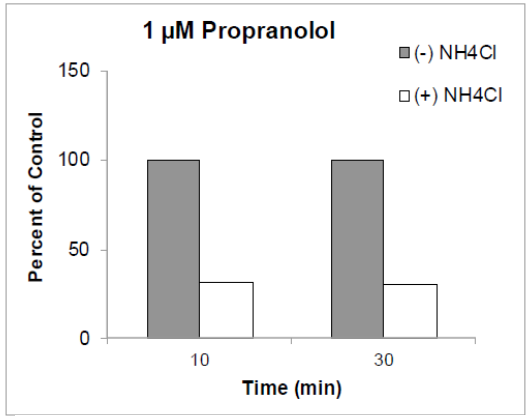
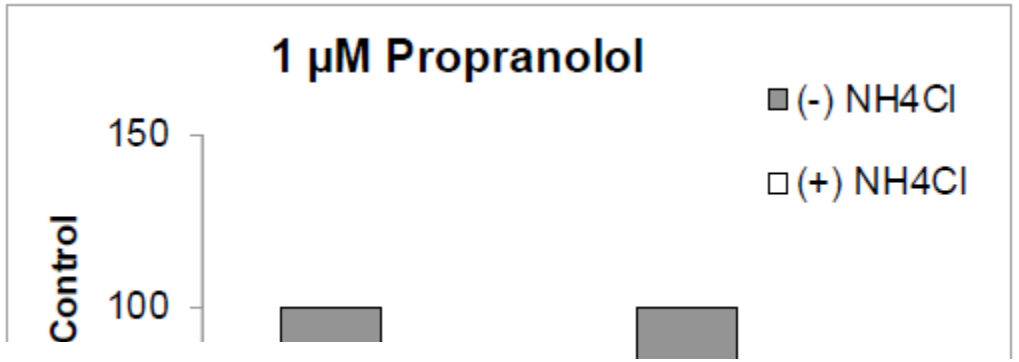
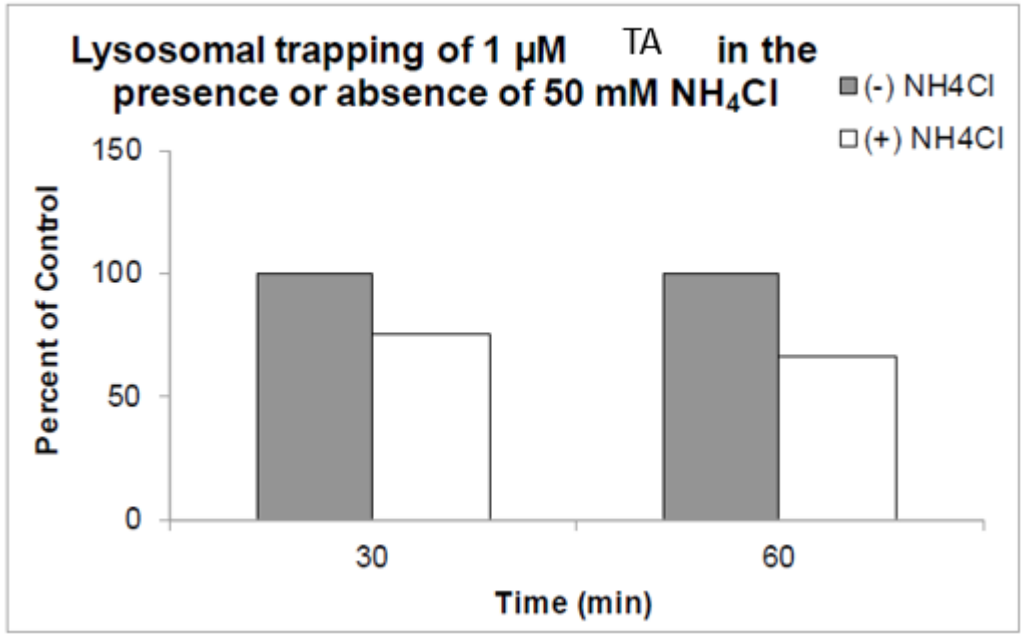
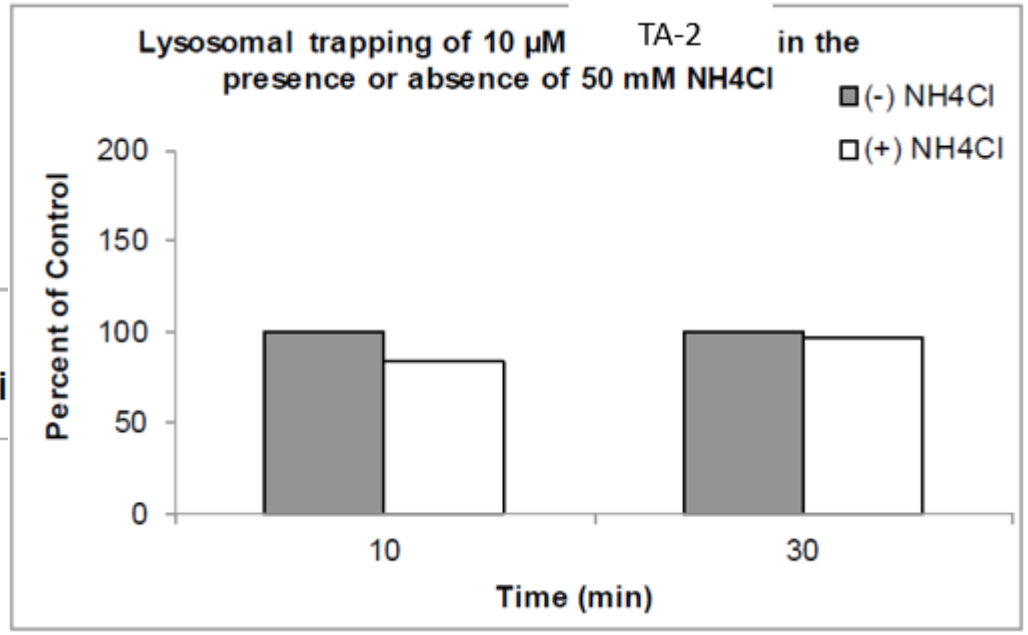
Mechanistic Assay for Lysosomal Trapping

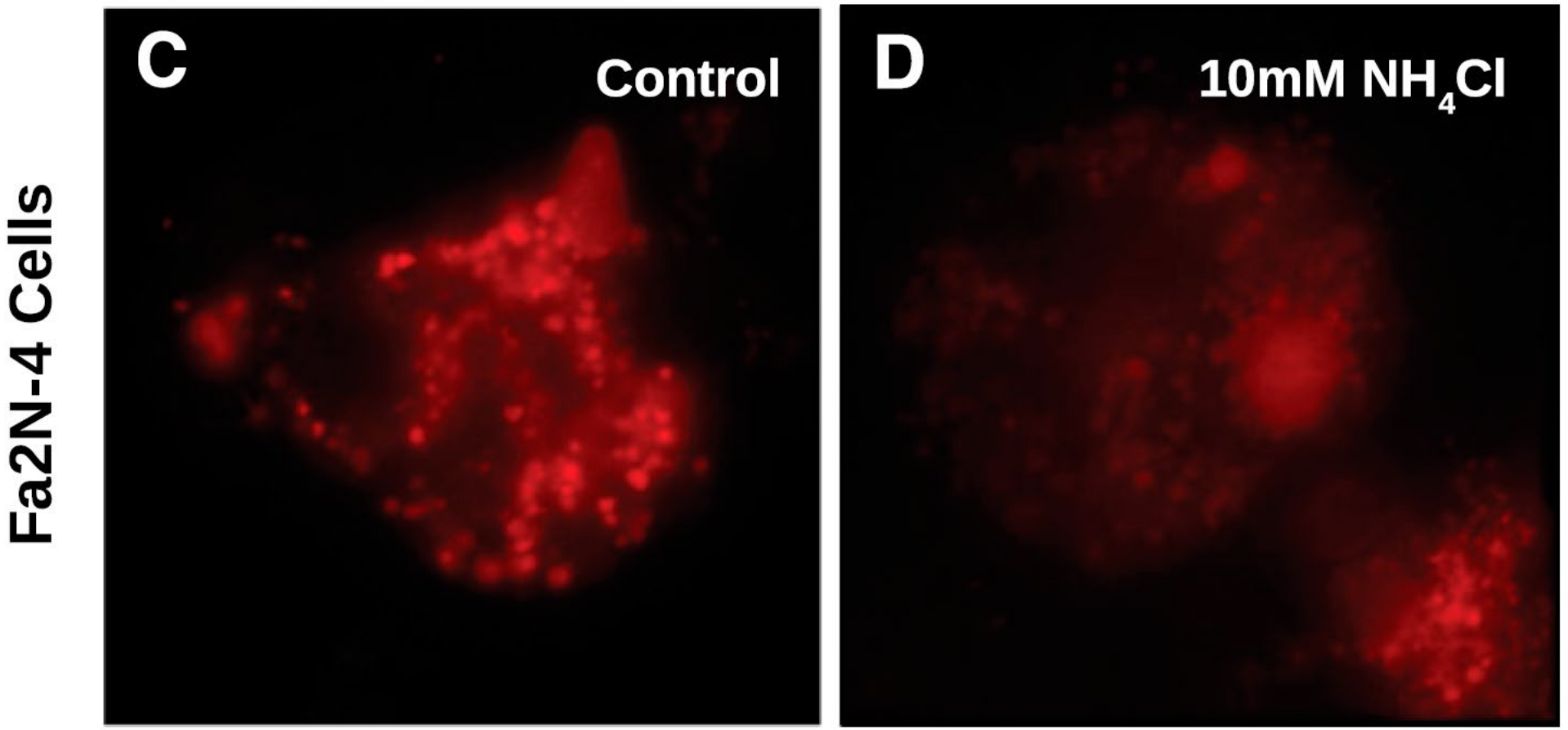








LysoTracker Assay for Lysosomal Trapping



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XenoTech Products Available for Lysosomal Trapping

Test System	Details	Supporting Reagents
Immortalized human hepatocytes (Fa2N-4)	• Plated on 6, 12, 24, 48 or 96 well collagen-coated plate • Consistent results, no lot-to-lot variability	• Proprietary nutrient-rich media containing phenol red that is supplemented with serum (component B) prior to use • Used for thawing, isolating, and seeding Fa2N-4 cells • 100 mL, 500 mL, or 1 L volumes
Primary human hepatocytes	• Cryoplateable • 4 or 6 mil AMY • Maintain enzyme and transporter activity	• OptiTHAW • OptiPLATE • OptiCULTURE


Cell & Tissue-Based Products
Radiolabeling
in vitro ADMET & Pharmacology
Metabolite ID & Production
Screening
API Manufacturing
in vivo ADMET & QWBA
Bioanalytical

Thank you!