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Live Cell Organelle-Specific Characterization of Cathepsins by Activity-Based Protein Profiling

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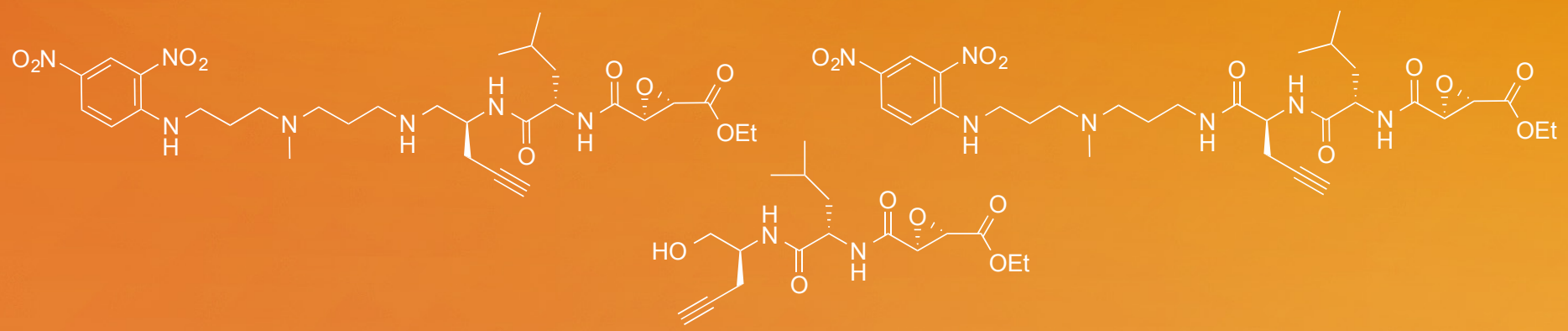
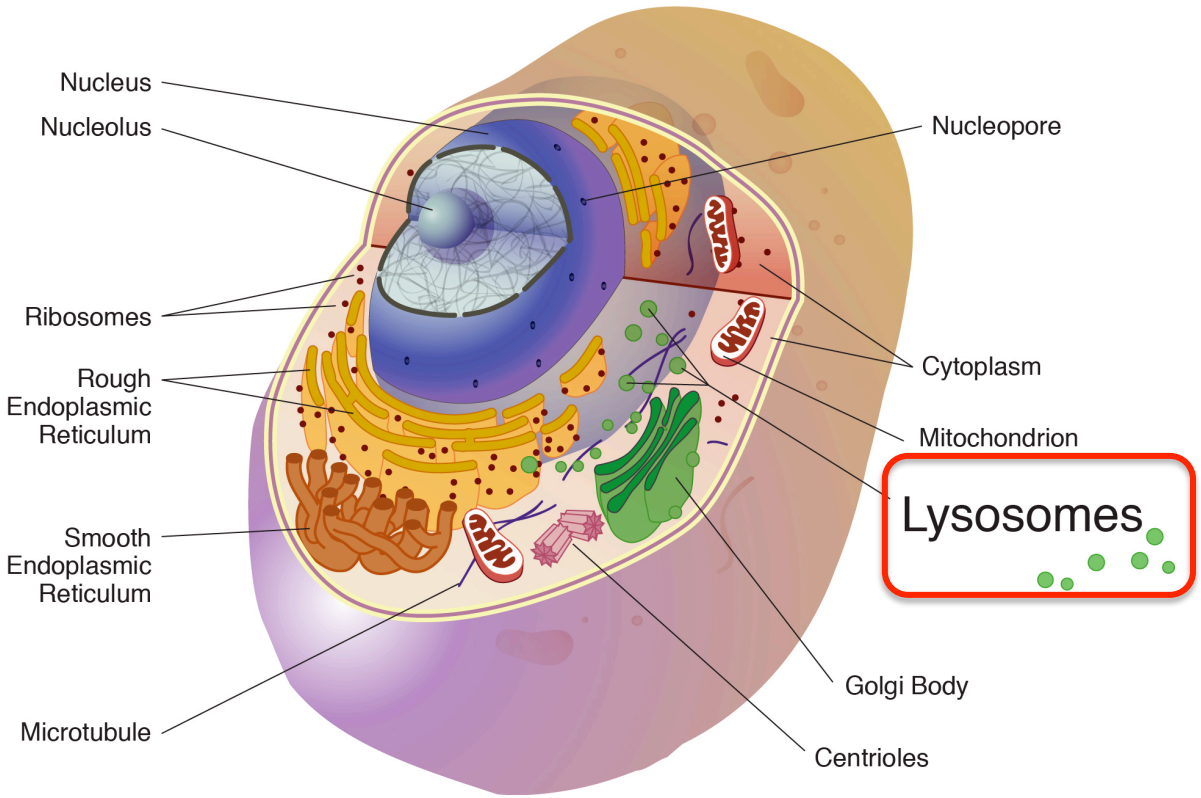


Live Cell Subcellular Targeting of Function



"Fluorescent sensors that can localize in a certain cellular region or organelle are highly suited to [analyzing the spatial and temporal dynamics of nucleoside polyphophates] because subcellular localization allows selective monitoring of biological events occurring in a specific region with high accuracy and low background." – Kurishita, Y. et al. *J. Am. Chem. Soc.* **2012**, *134*, 18779.

Live Cell Lysosome Targeting of Protein Function

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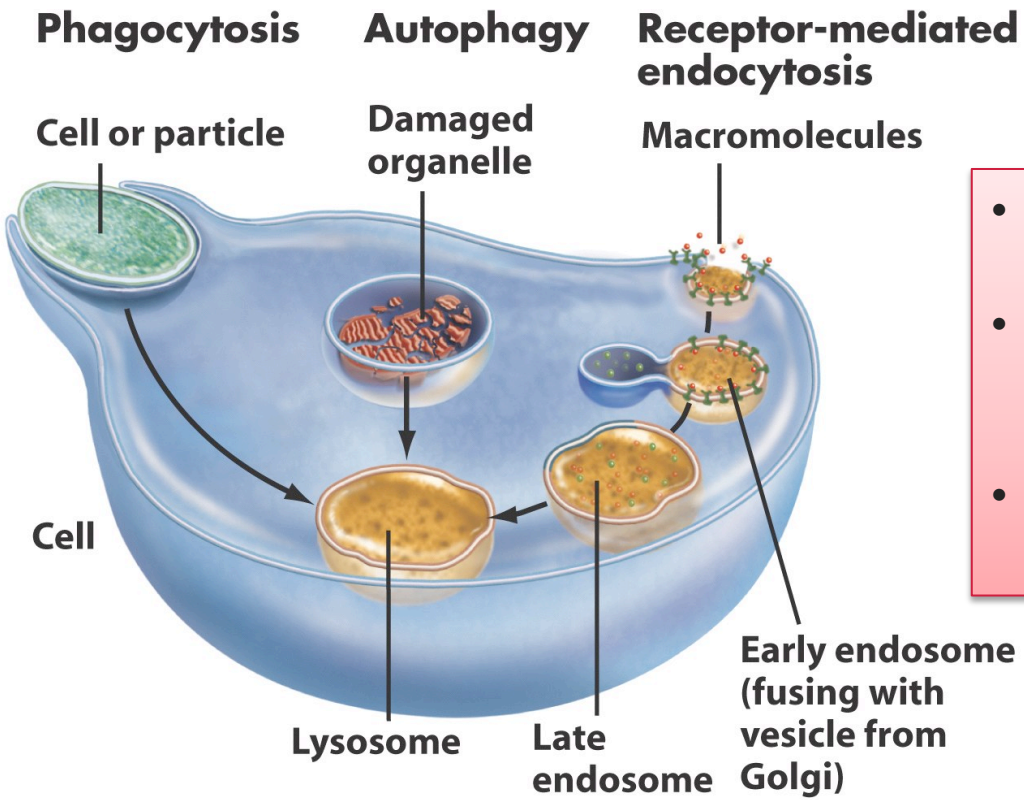
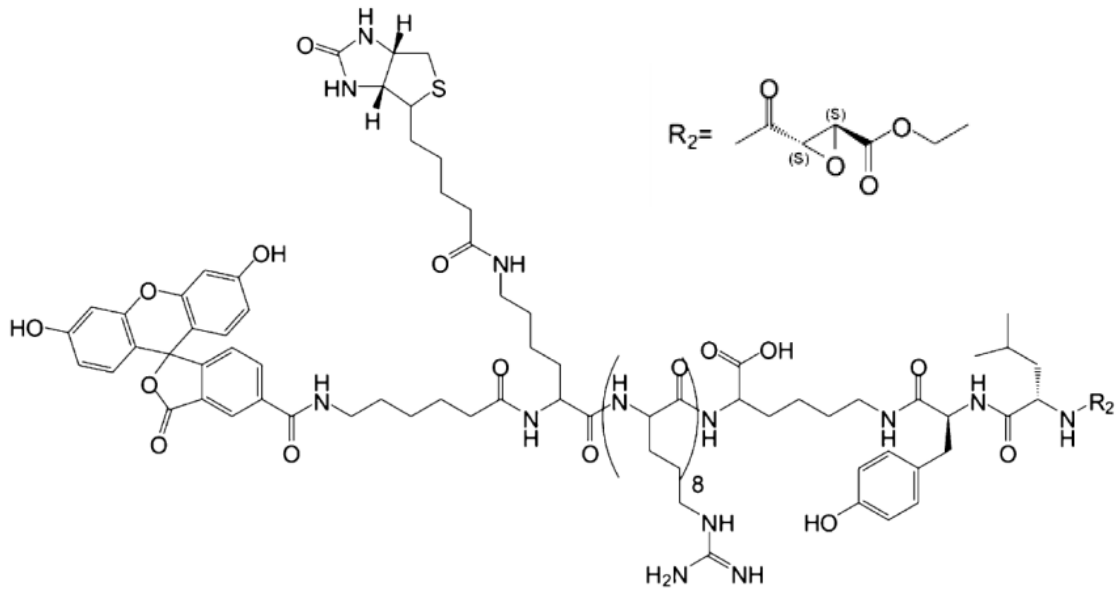


- Internal acidic pH (~4.5-5.0)
- Contains >60 catabolic enzymes (e.g., cathepsins, caspases, various hydrolases)
- Associated with numerous diseases, including >50 'orphan' diseases

A lysosome is a membrane-bound cell organelle that contains digestive (catabolic) enzymes. They are involved in several cell processes including: (1) the break down of excess or worn-out cell parts; (2) the destruction of invading viruses and bacteria; and, (3) self-destruction of the cell in a process called programmed cell death, or apoptosis.

Prior Lysosome Targeting Approaches

- Enzyme selective: probe is selective for enzyme residing in the lysosome



Fan, F. et al. *J. Proteome Res.*, **2012**, *11* (12), pp 5763–5772

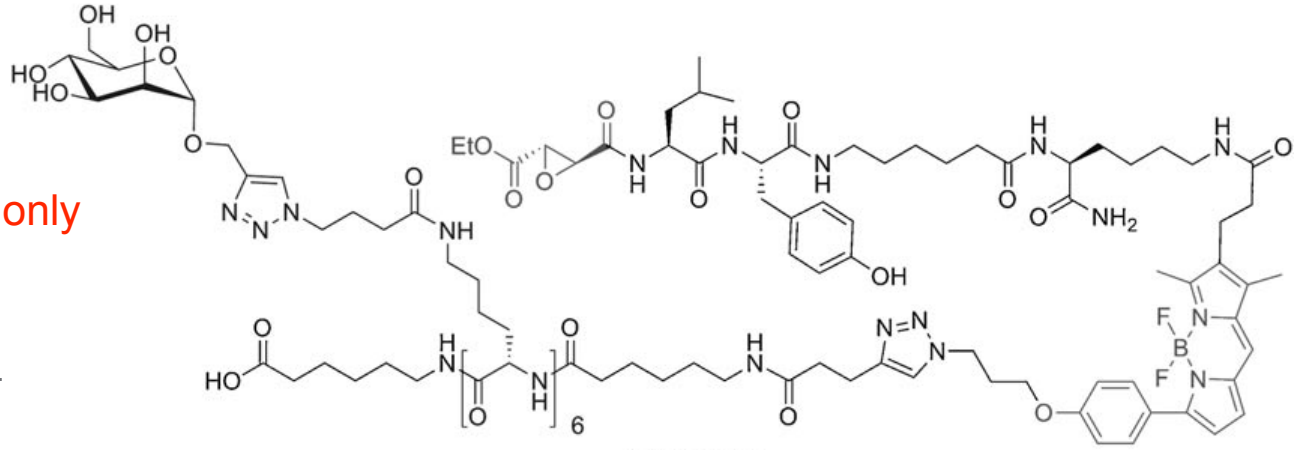
- Selective for cathepsins
- 4 h to accumulate in lysosome
- MW = 2639.39
- Cell Penetrating Peptide required

Prior Lysosome Targeting Approaches

- Endocytosis: uptake into endosomal/lysosomal pathway

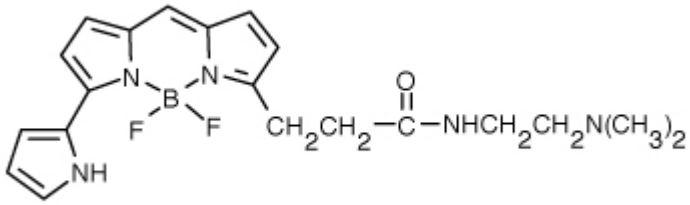
- Mannose Receptor uptake
- Designed for fluorescence only
- MW = 1399.5
- Very long labeling time

Hillaert, U. et al. ACIEE **2009**, *48,* 1629.



2 (UHG392)

- Weakly basic amines



LysoTracker® Red DND-99
Molecular Probes

- Designed for fluorescence only
- Very short labeling times
- Basic amines are highly selective for lysosome

Activity-Based Protein Profiling to Characterize Biological Function

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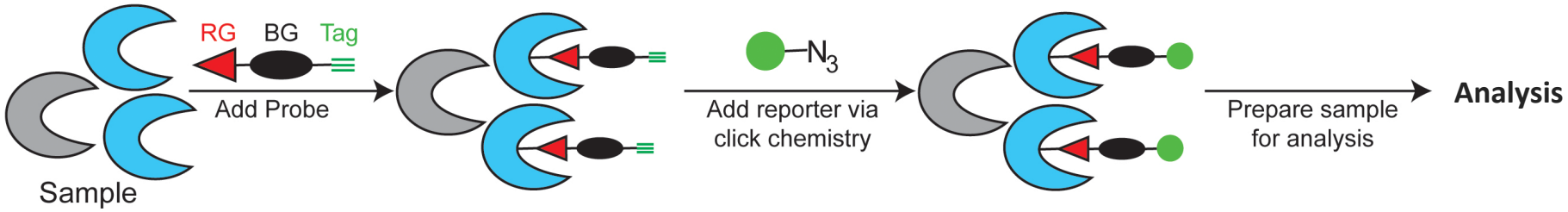
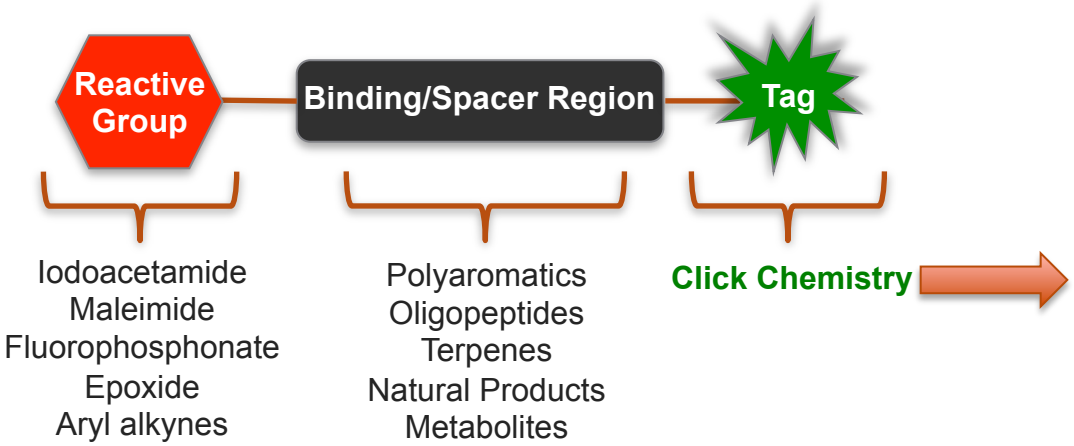


Activity-based protein profiling (ABPP), relies on active-site directed chemical probes to form irreversible covalent bonds to enzyme targets in complex proteomes, and subsequently report on their functional activity using LC-MS based proteomics, fluorescent SDS-PAGE, or microscopic imaging.



We can append nearly any fluorophore or enrichment moiety (e.g., biotin) with a bio-compatible Cu-catalyzed reaction.

Designing Activity-Based Probes for Cathepsins in the Lysosome

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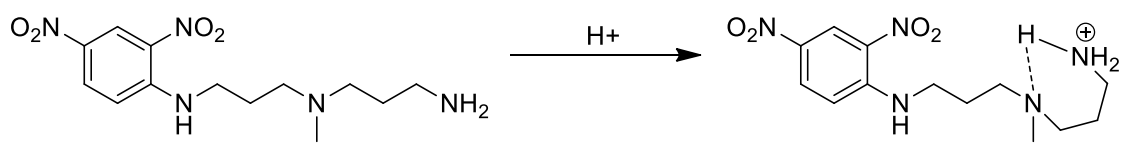
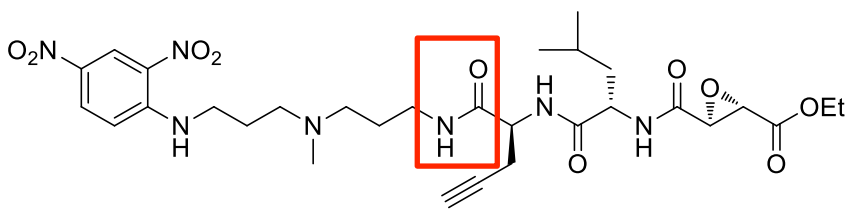
- Targeted cathepsins, which are cysteine proteases involved in lysosomal catabolic activity.
- We took advantage of the acidic environment of the lysosome.
- We designed a series of probes from a known cathepsin inhibitor, E64D.
- **We wanted fast labeling times, lysosome selectivity, and multimodal analyses.**



3-(2,4-dinitroanilino)-3'-amino-*N*-methyldipropylamine

DAMP accumulates in the lysosome



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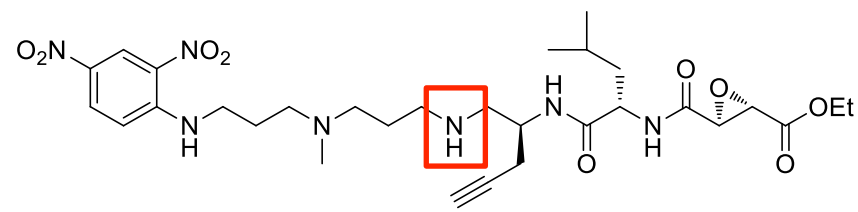

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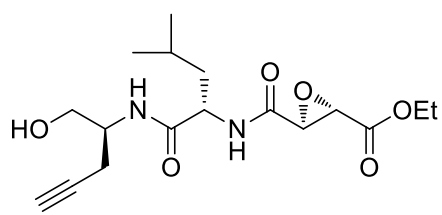

DEX-3

Wiedner SD, et. al. *Angew. Chem. Int. Ed.* **2014**, *53*, 2919-2922.

Global Cell Lysate Labeling With DEX Probes

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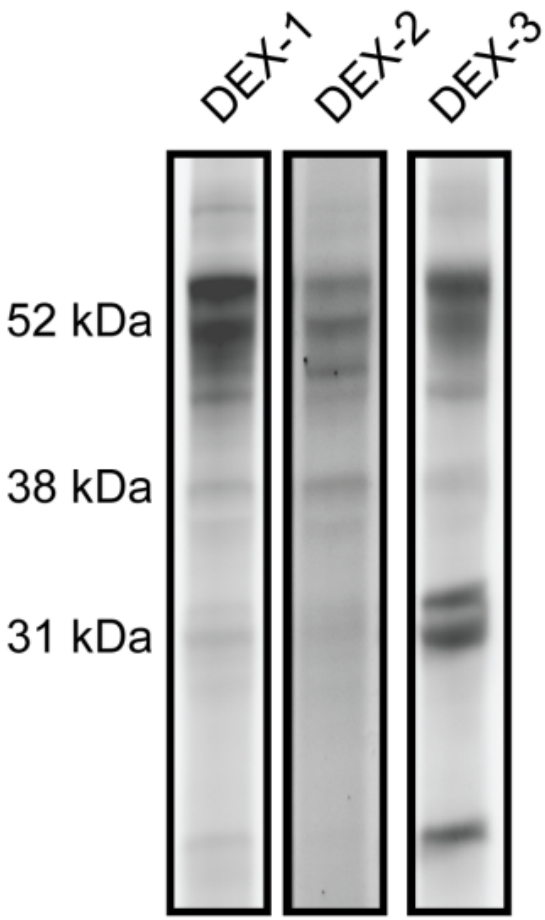
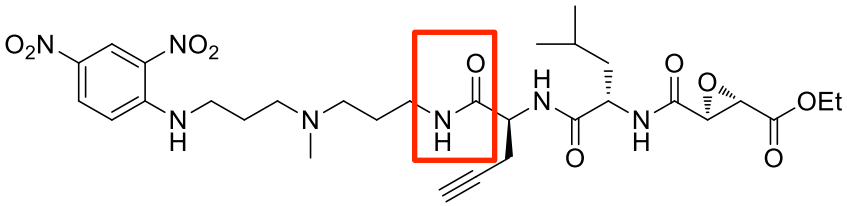


A549 global cell lysate labeled with DEX probes (50 μM)



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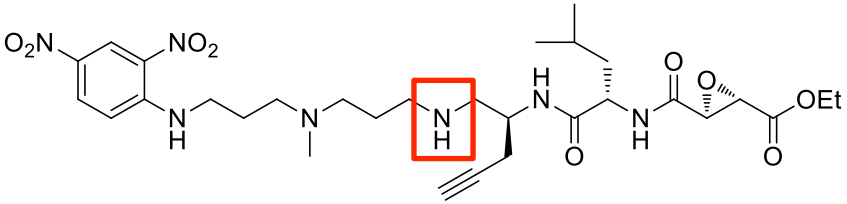

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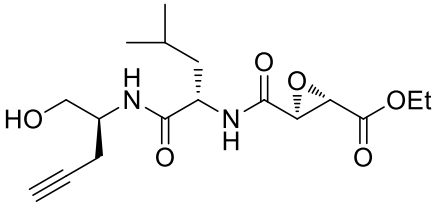

DEX-3

Wiedner SD, et. al. *Angew. Chem. Int. Ed.* **2014**, *53*, 2919-2922.

A549 Lysosome Labeling – Probe Selectivity

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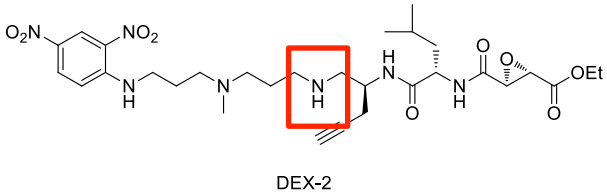

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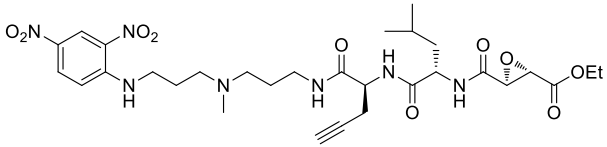

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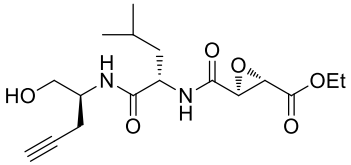

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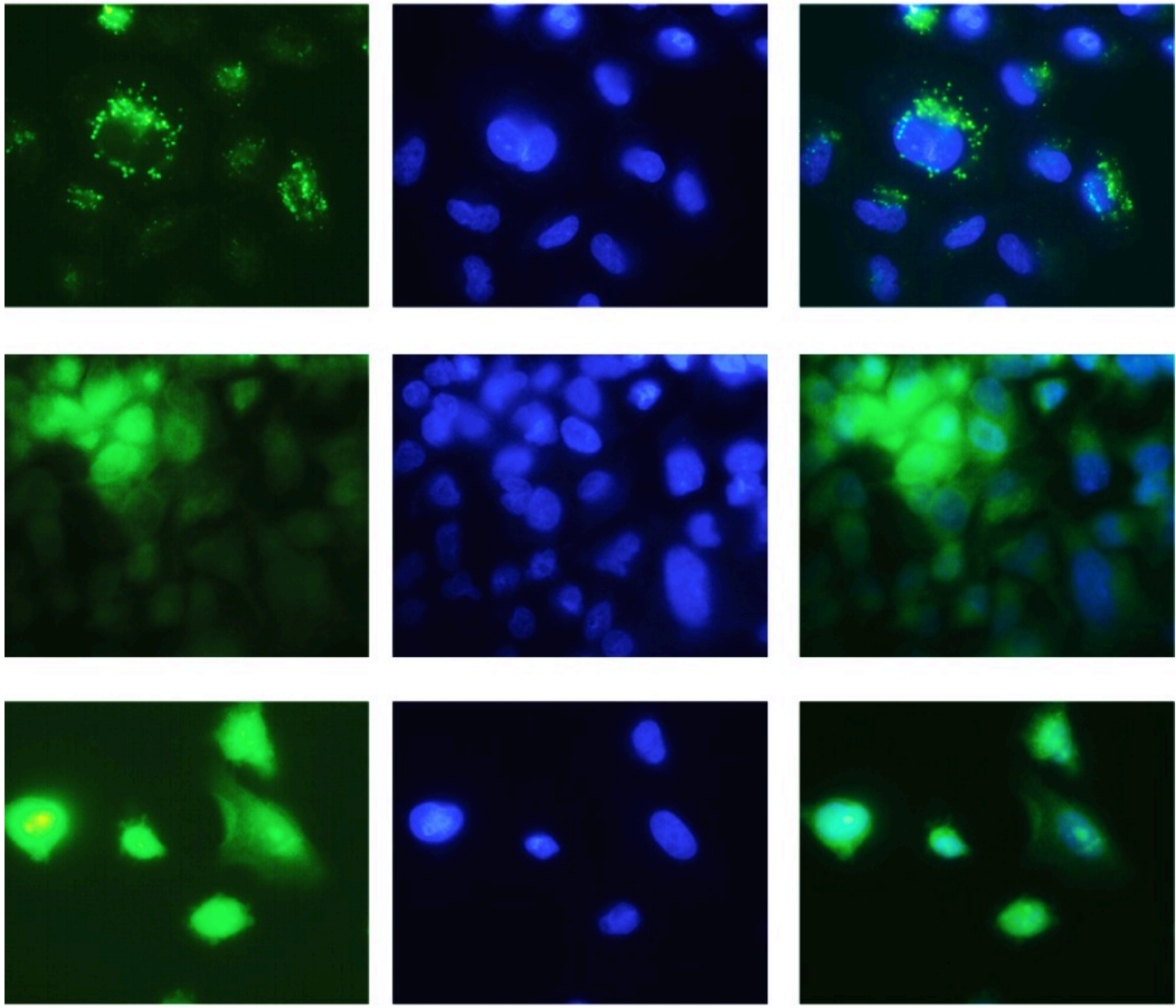

The secondary amine of **DEX-2** permits rapid organelle entry.

Speed of Accumulation of DEX-2

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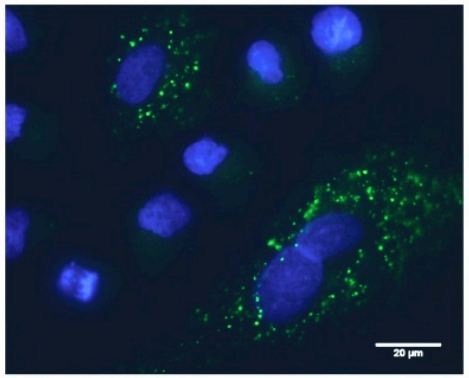
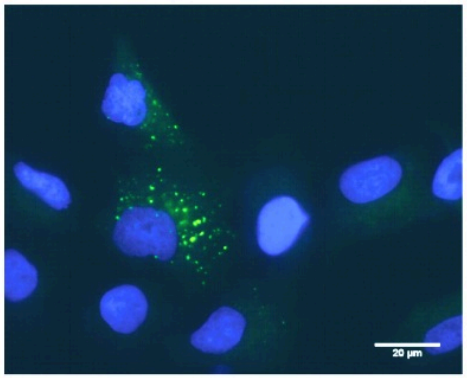
A

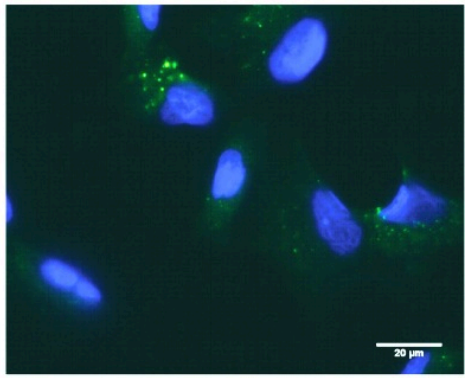
60 min



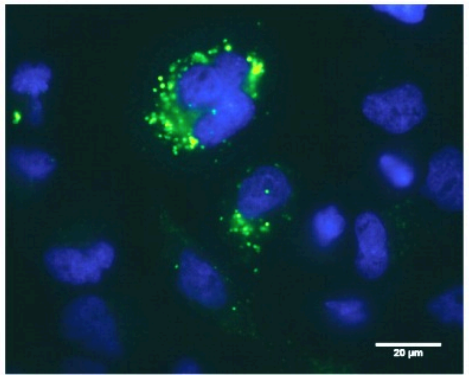
30 min



10 min



B



(A) A549 cells were treated with **DEX-2** (10 µM) for 60 min, 30 min, and 10 min and then fixed with 3.6% paraformaldehyde and stained with AlexaFluor 488 ClickiT Kit (Invitrogen).

(B) A549 cells were treated with **DEX-2** (10 µM) for 60 min and then washed with probe free media for 4 h and visualized as above.

Confocal Laser Microscopy - Organelle Markers & DEX-2 Labeling of A549 Cells

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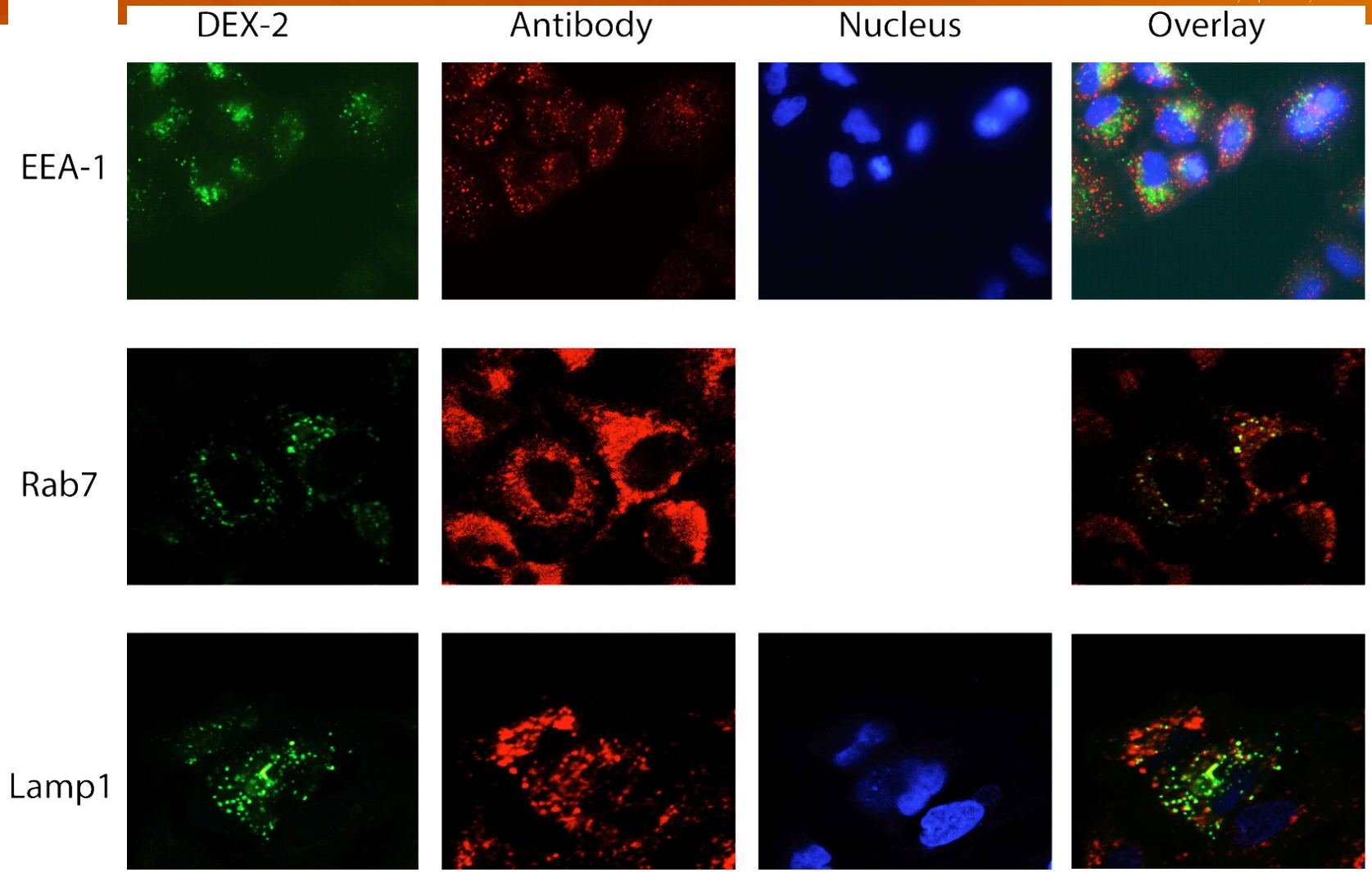

- Does not co-localize with early endosome (EEA1)
- Is also closely associated with the late endosome (Rab7)
- Is closely associated with the lysosome (Lamp1)

Super-Resolution Structured Illumination Microscopy – J774 Macrophage Cells Labeled with DEX-2

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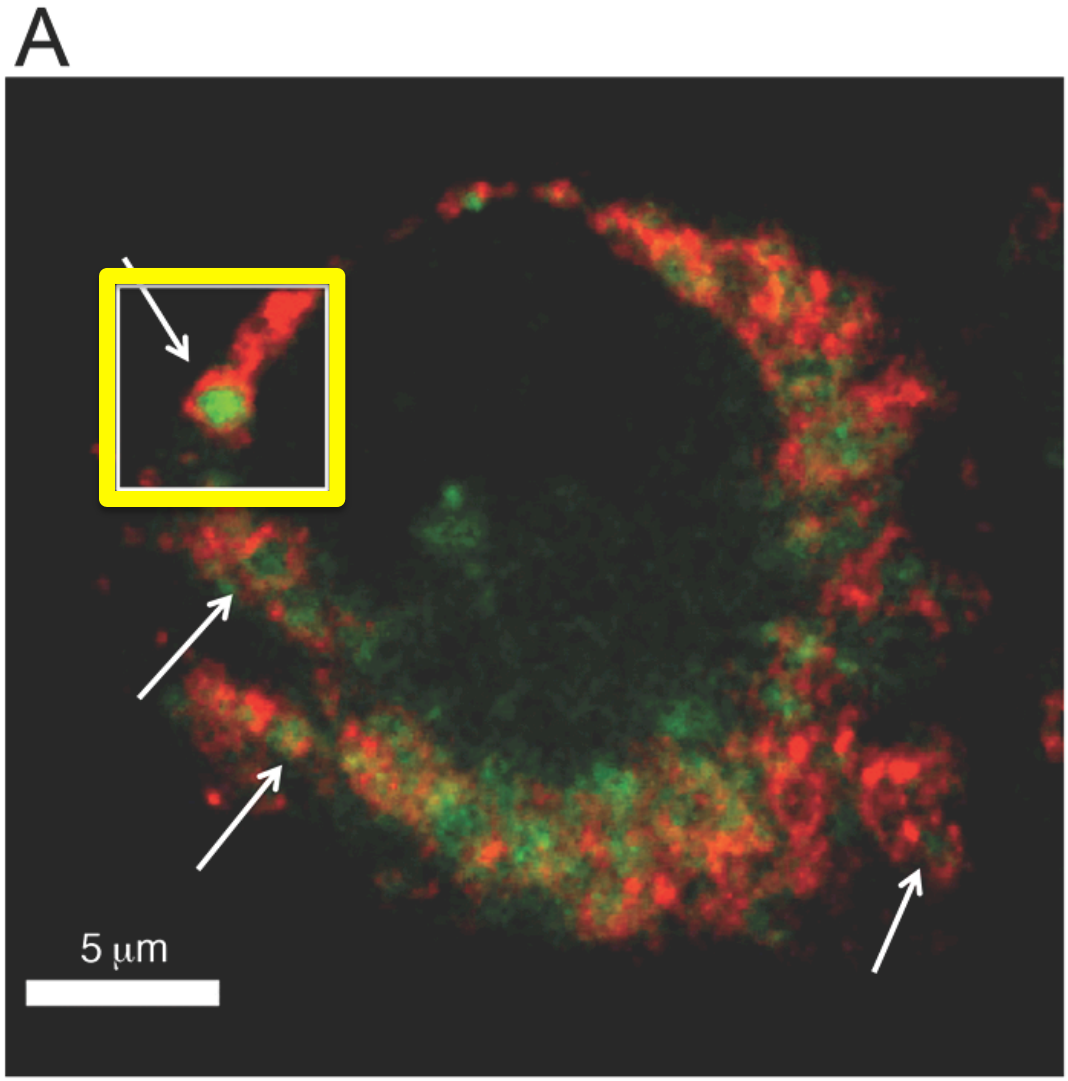

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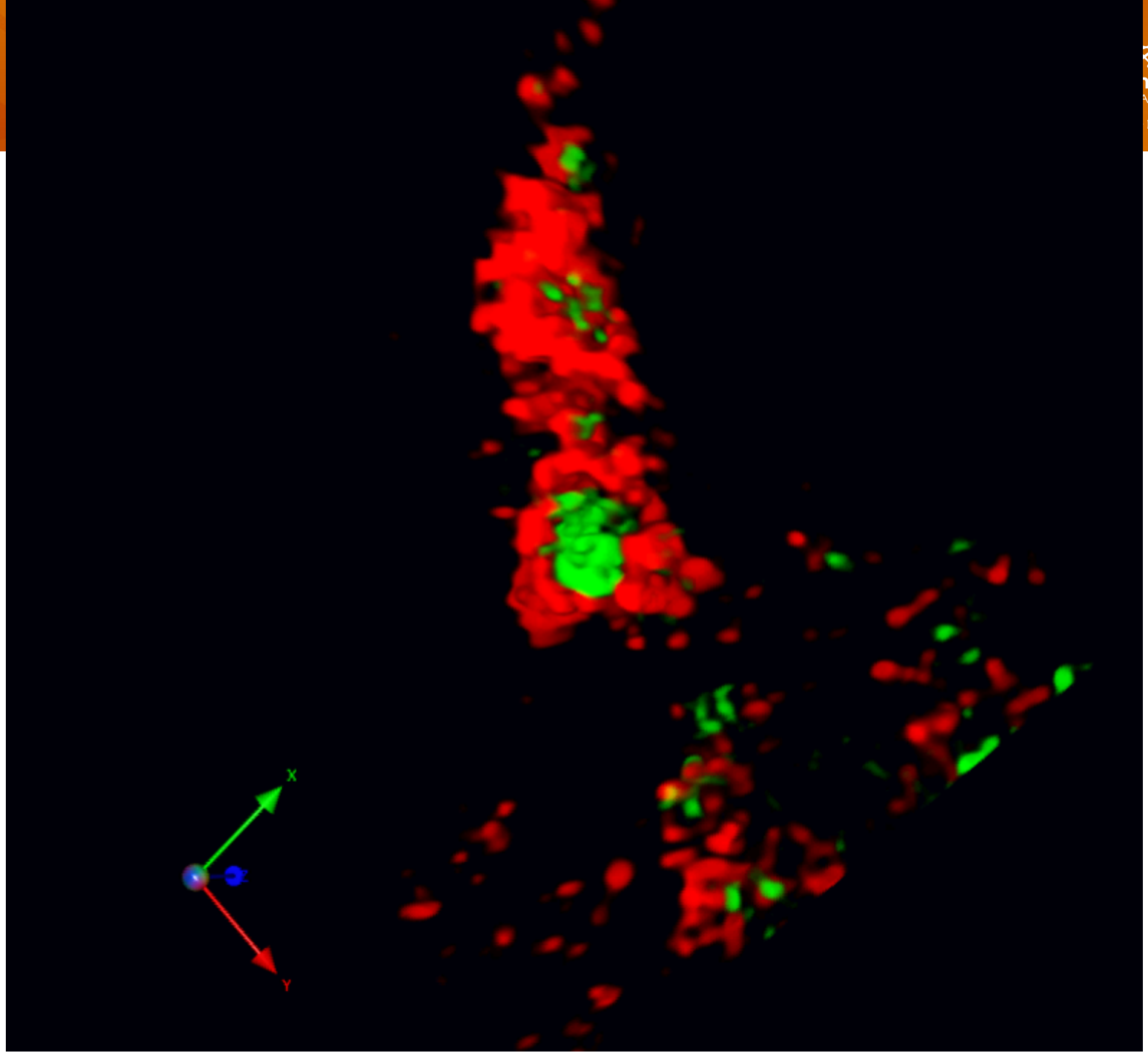
X
Y

LC-MS Analysis of Autophagy in J774 Macrophage Cells

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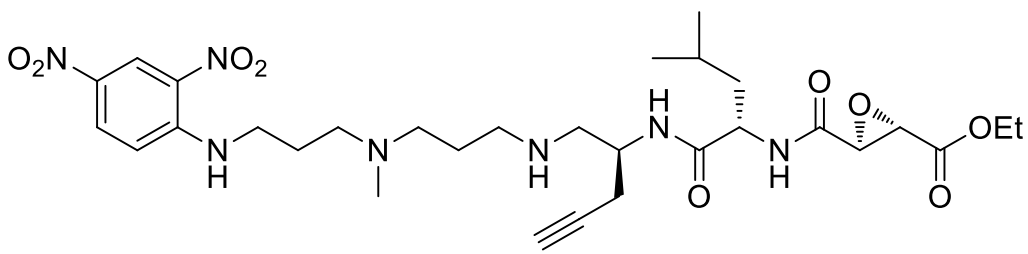
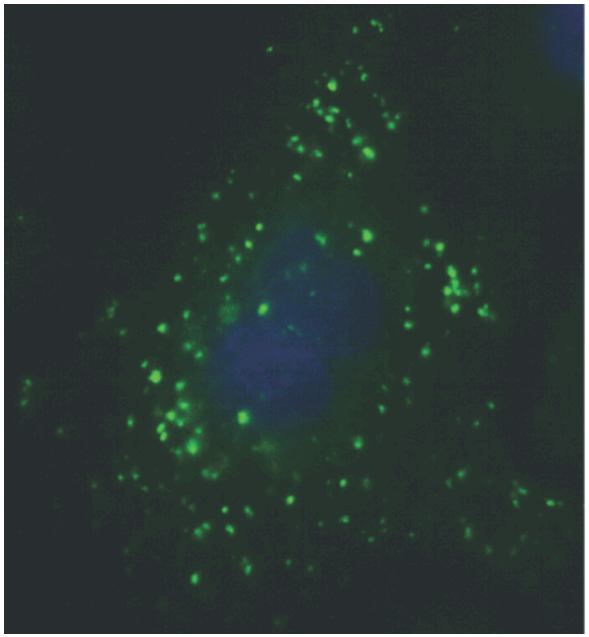
Autophagy is a catabolic mechanism in which cell degradation of unnecessary or dysfunctional cellular components occurs through the actions of the lysosome.

- Exquisite selectivity observed in healthy cells when comparing probe labeling to control samples. Only six targets were observed by the probe, with the dominant targets being CatB and CatZ (caspases). FDR ~1%.

- Upon induction of autophagy probe labeling increased to 44 proteins, 17 of which are previously characterized as lysosomal proteins during autophagy. FDR ~1%.



DEX-2



Wiedner SD, et. al. *Angew. Chem. Int. Ed.* **2014**, *53*, 2919-2922.

Summary & Future Directions

- Tuning the physicochemical properties of a small molecule can result in an effective probe for characterizing specific enzyme activities in defined subcellular locales.
- Changing the reactive group attached to the DAMP moiety will allow us to target other catabolic enzymes in the lysosome.
- Can we identify chemistries to target enzyme populations within other organelles?

Acknowledgements

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- Dick Smith
- Ron Moore
- Karl Weitz

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EMSL

NIH National Institutes of Health