Routes of Cellular Uptake of Nano-sized Materials Depend on Compositions of Cell-Penetrating Peptides

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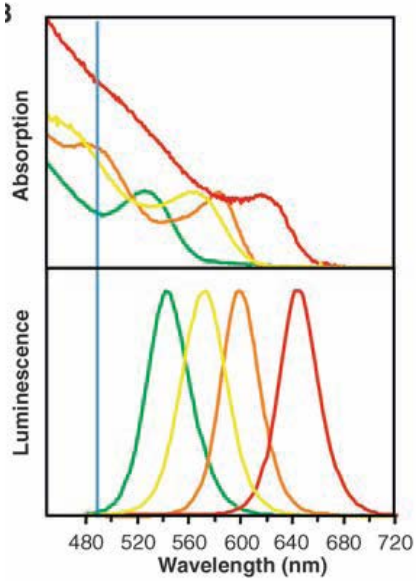
Organization of this Presentation

- Cd-based quantum dots (QDs)
- Cell-penetrating peptides (CPPs)
- Delivery of QDs by CPPs
  - Uptake mechanisms (why important?)
    - Pharmacological inhibitors & siRNAs
- Conclusions

Cd-based Quantum Dots – QDs

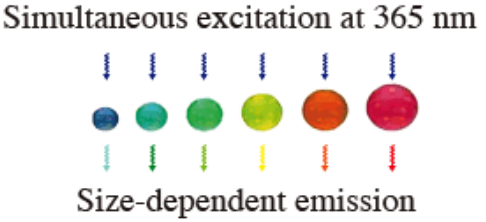
(Unique Optical Properties)

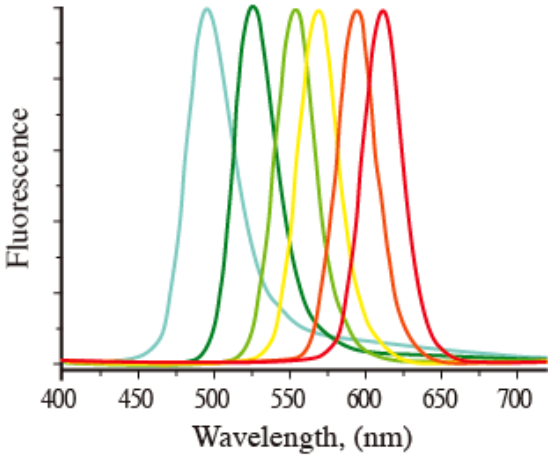


Broad absorption spectra

Symmetric, narrow emission spectra

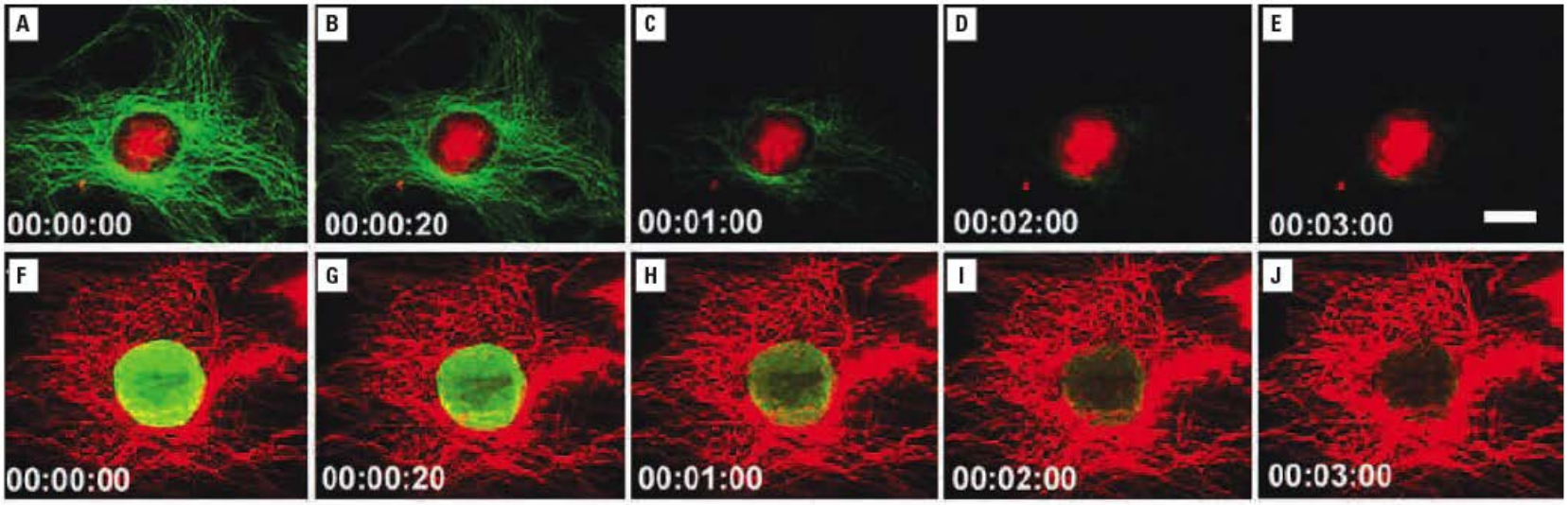
Size-dependent fluorescent colors



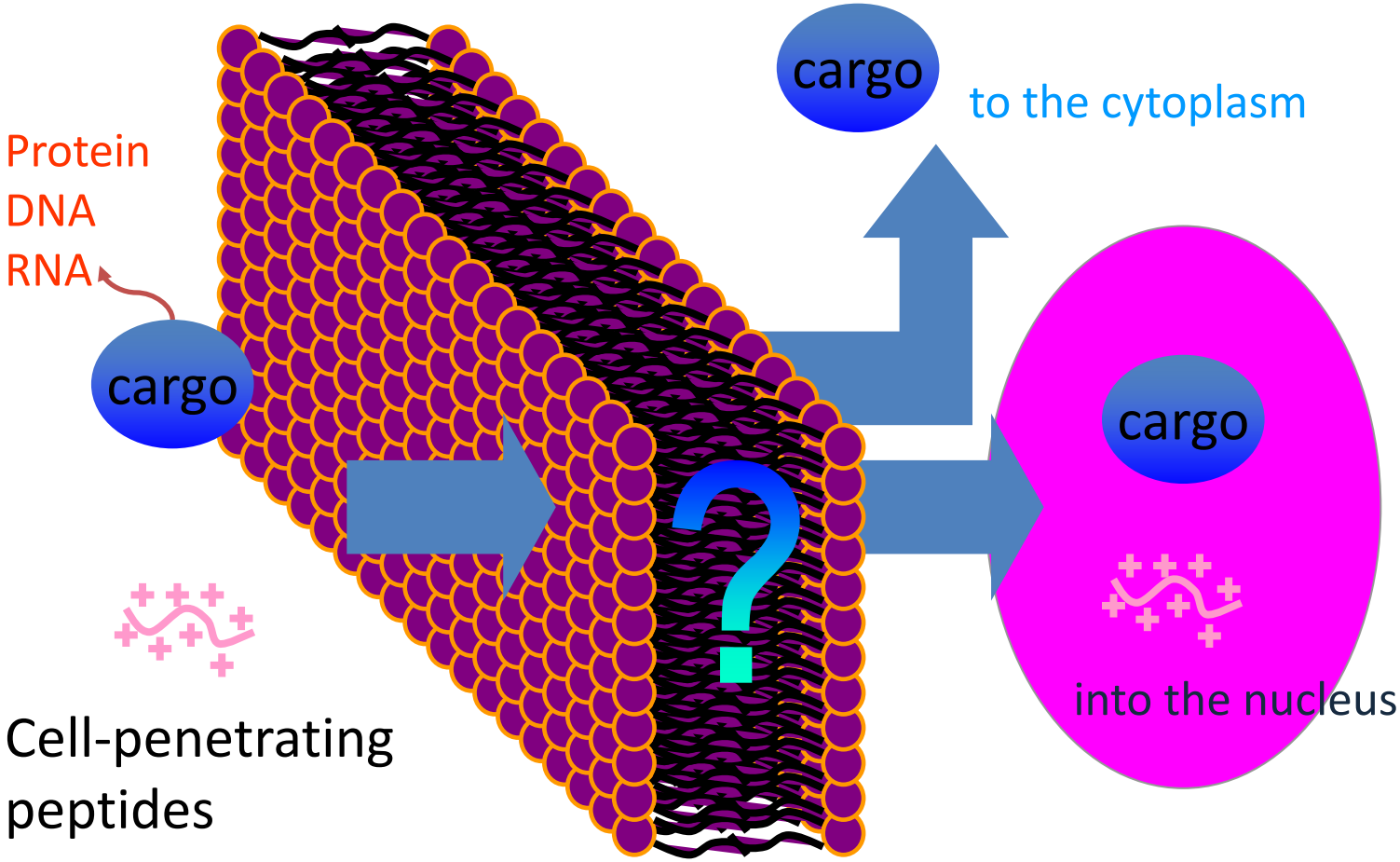


Unique Optical Properties of QDs

- Photostability

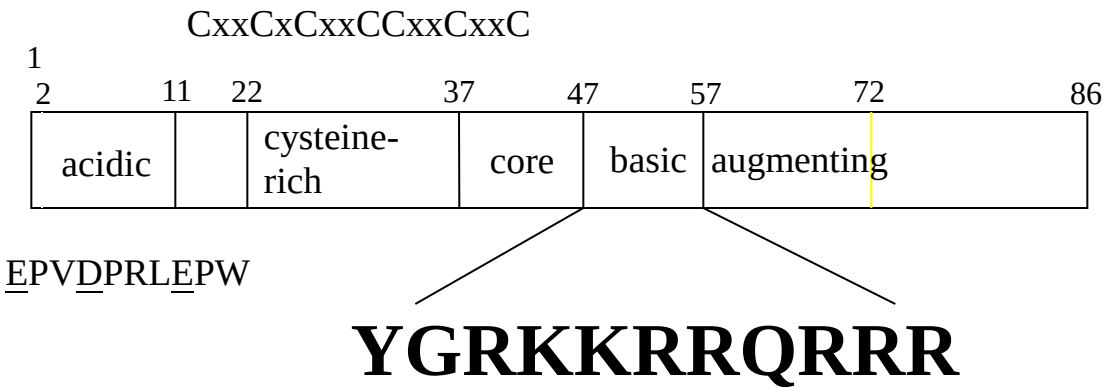


Cell Membrane as a Selective Barrier



Protein Transduction Domain (PTD)

- Functional domains of HIV-1 Tat:



Basic amino acid-rich domain

Gaynor, Transacting functions of human retroviruses p.51-77, 1995

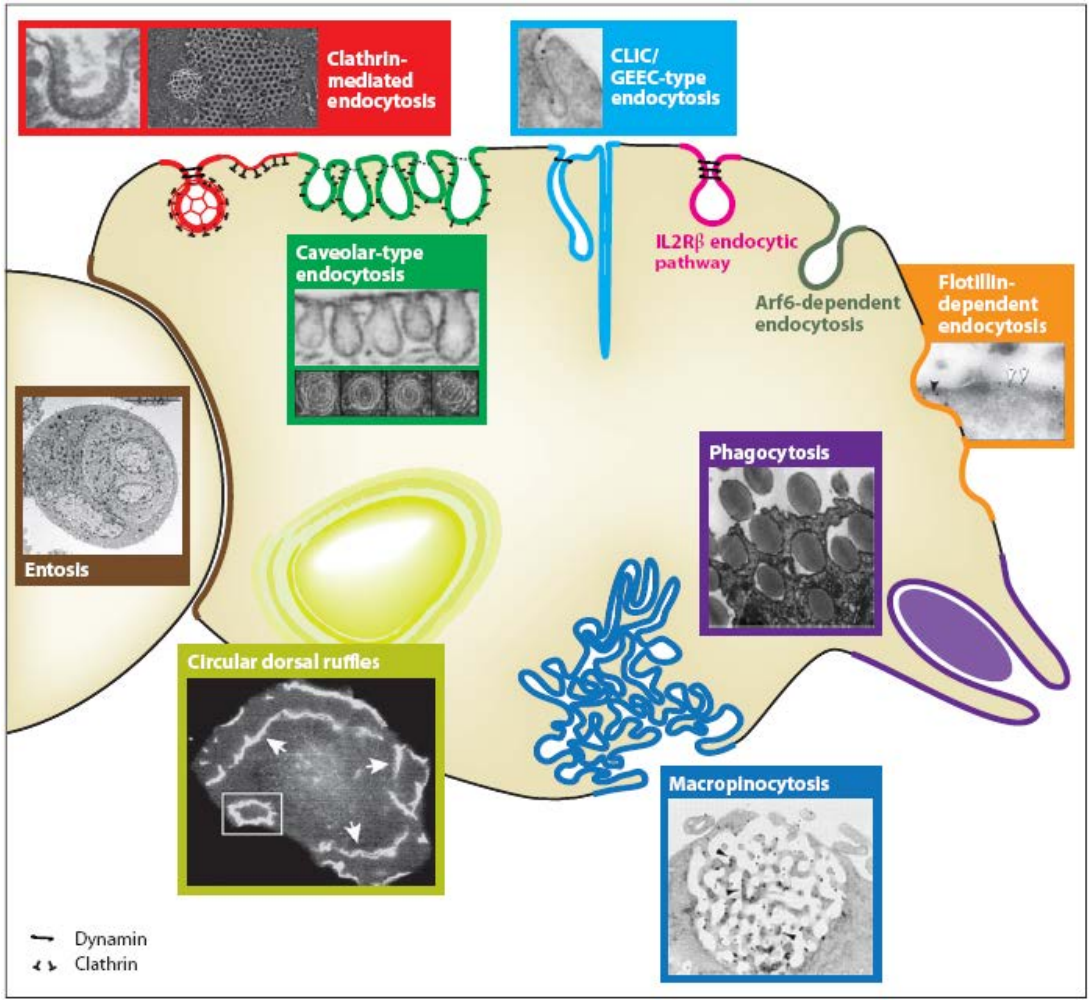
Cell-Penetrating Peptides (CPPs)

- < 30 amino acids
- Rich in arginine and lysine
- Positively charged; amphipathic
- Easy preparation; low cytotoxicity
- High protein transduction efficiency

CPP Uptake Mechanisms

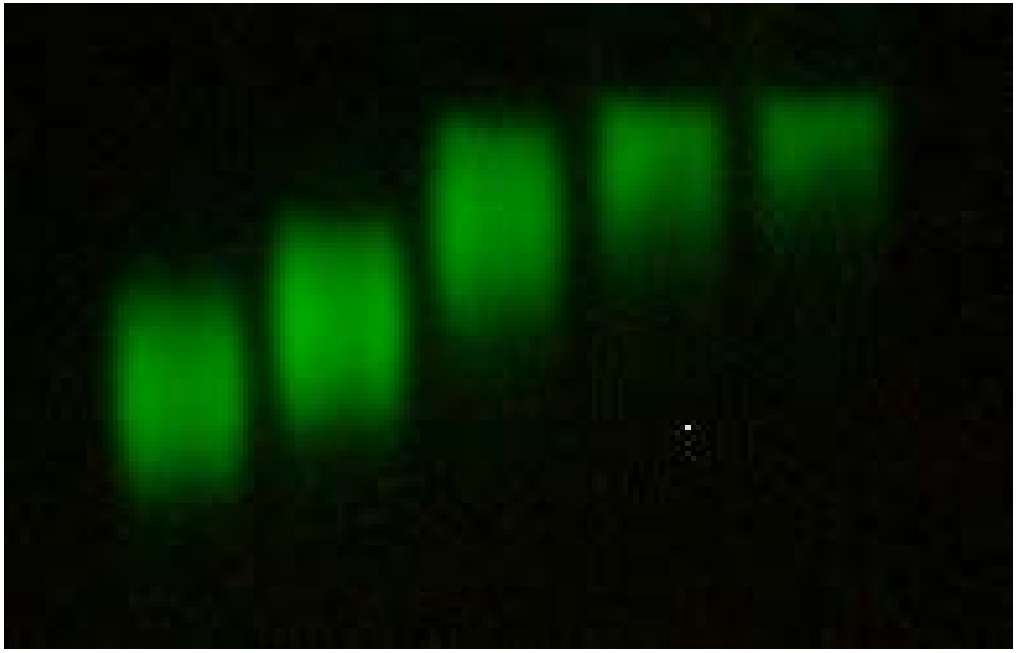
- Direct Membrane Penetration
  - Inverted micelle
  - Carpet-like model
  - Membrane thinning model
  - Pore formation
- Endocytosis

Which Endocytic Pathways?

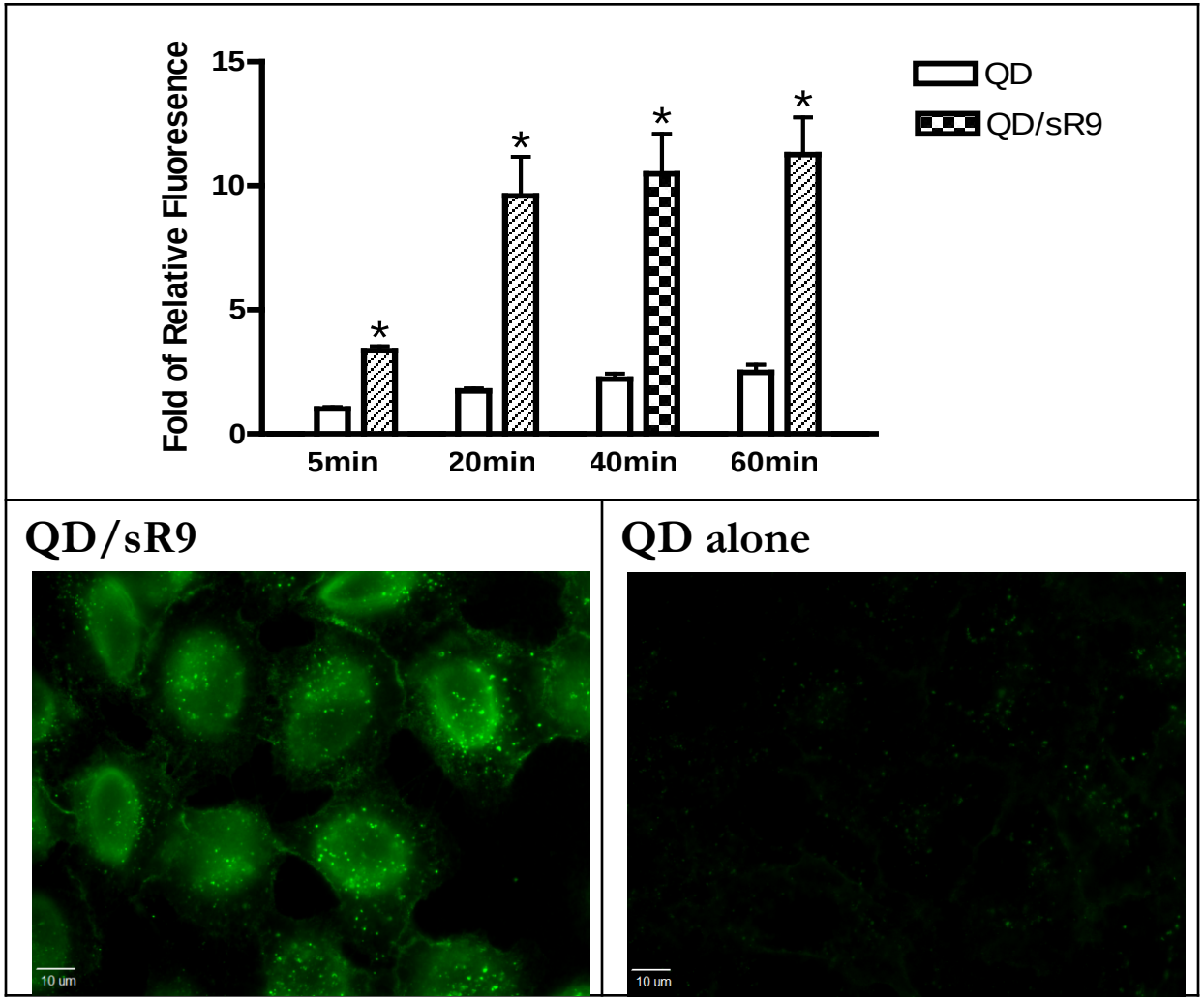


Annu. Rev. Biocehm. 2009. 78:857-902.

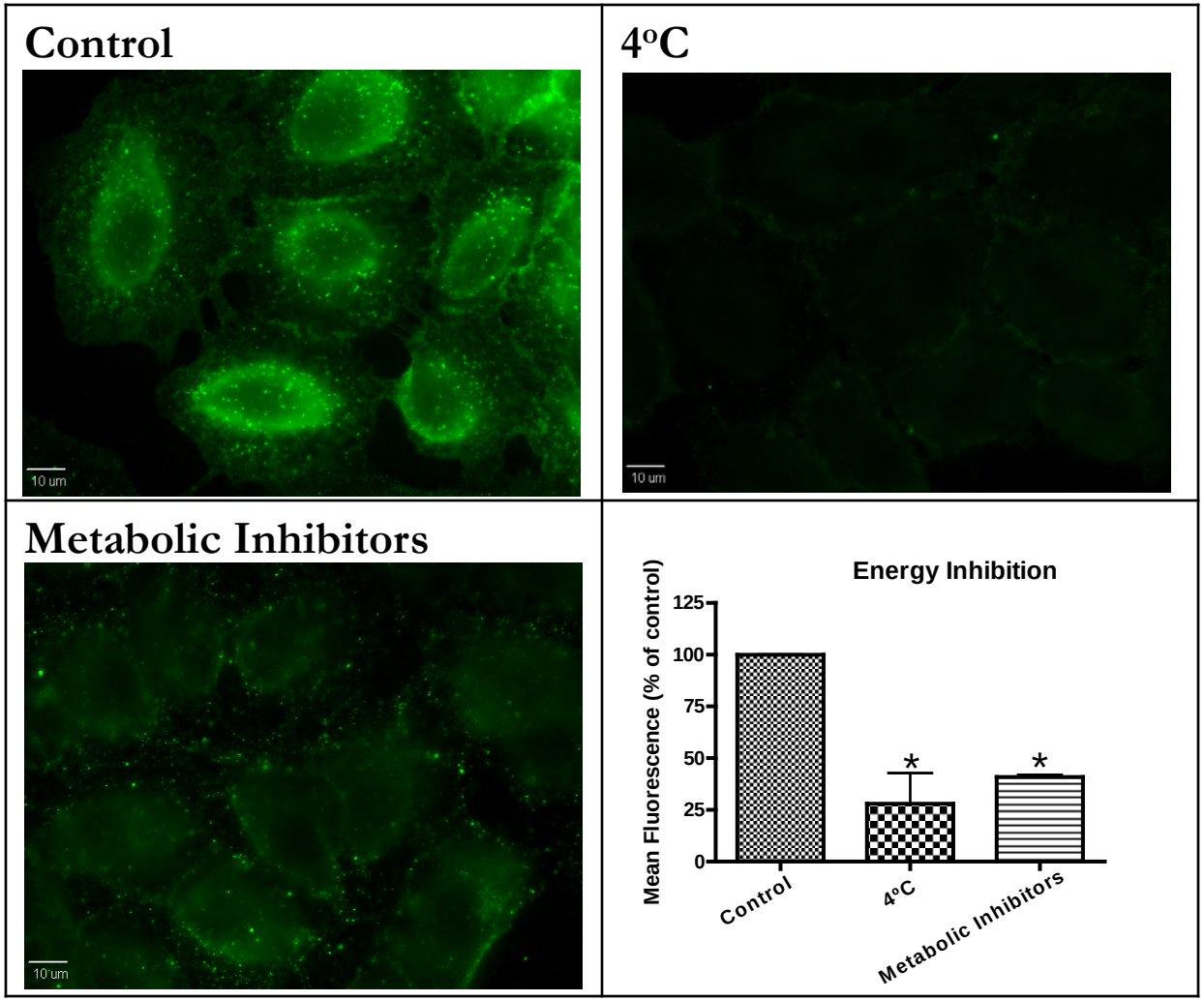
Noncovalent Interaction of QD and sR9



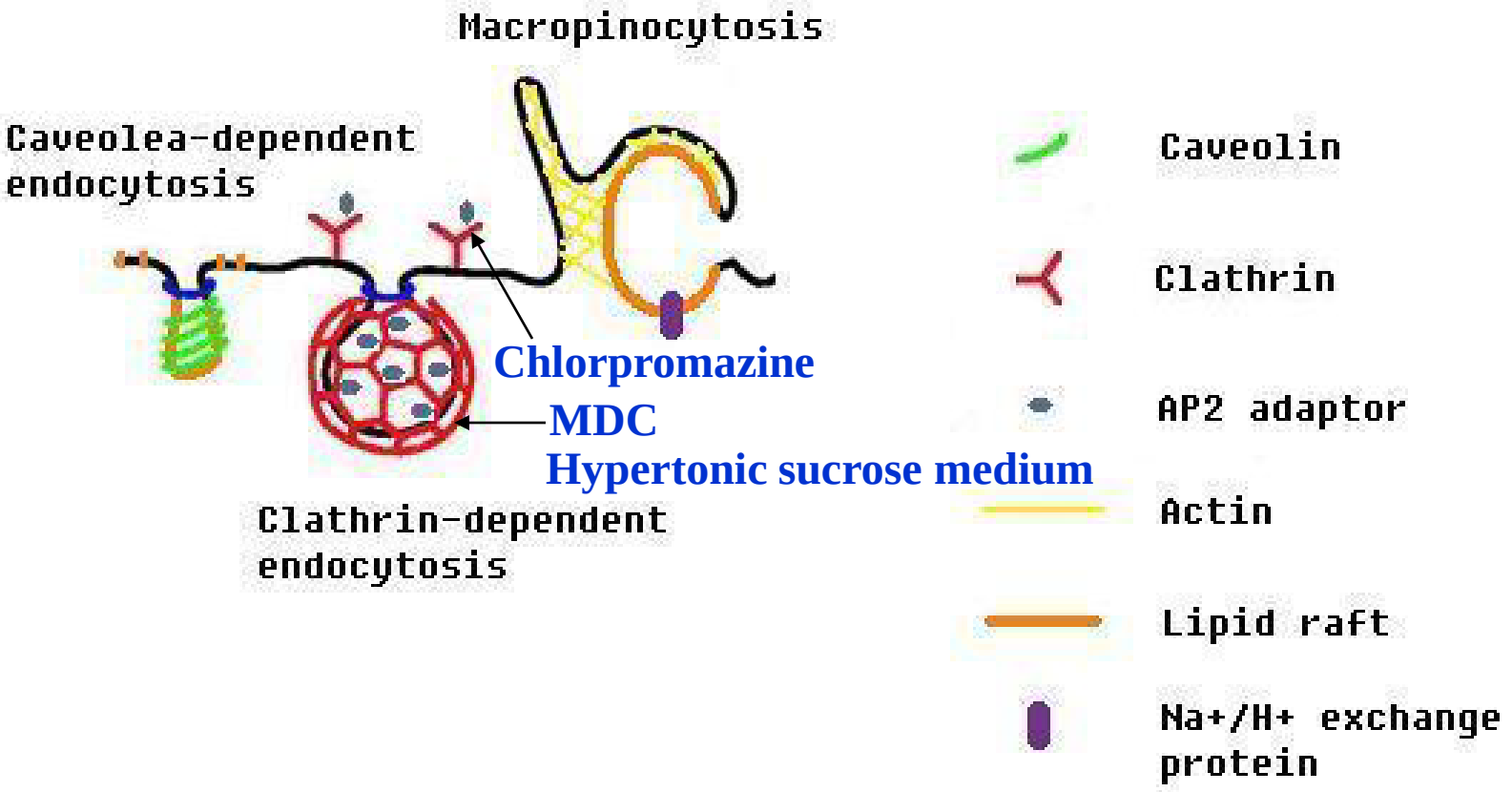
sR9 Facilitates QD Uptake



Uptake is Energy-dependent

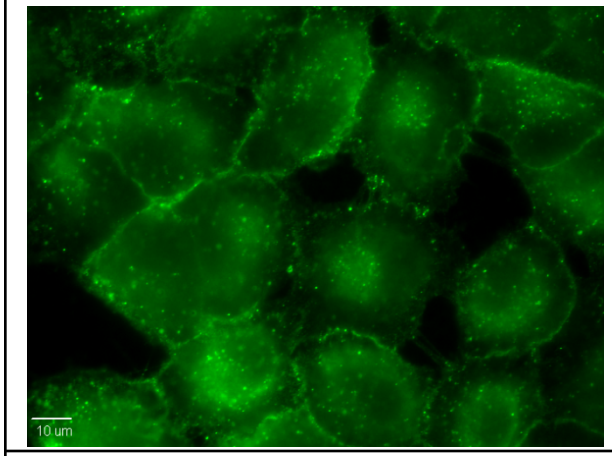


Clathrin-dependent Pathway

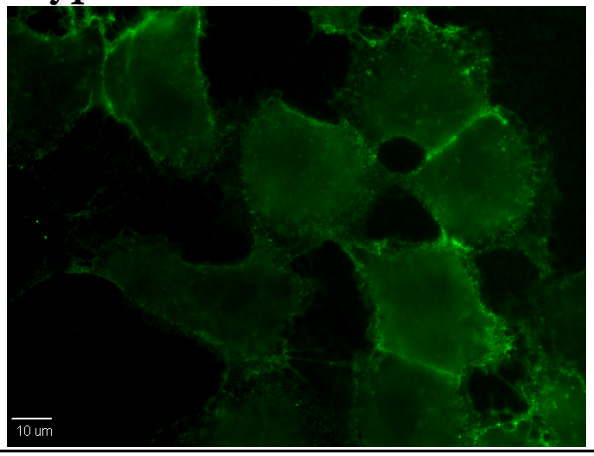


Differential Effects of Inhibitors on Clathrin-dependent Pathway

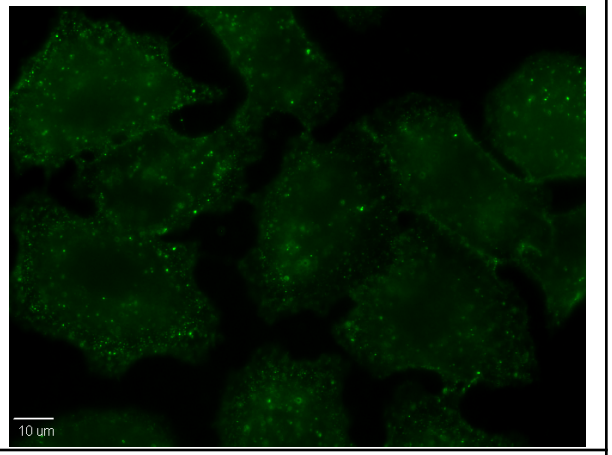
Control



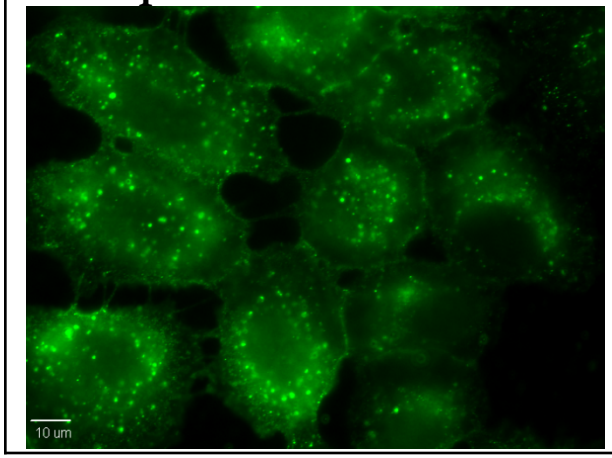
Hypertonic sucrose

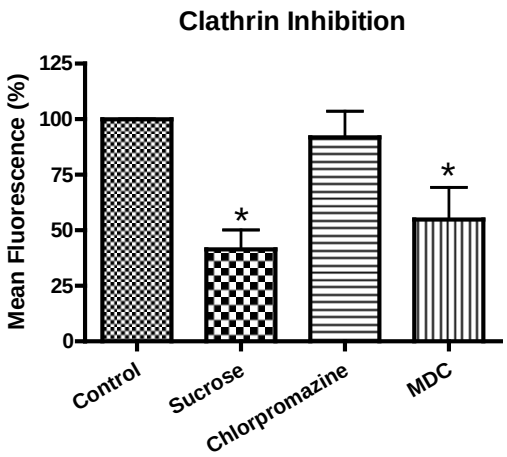


MDC

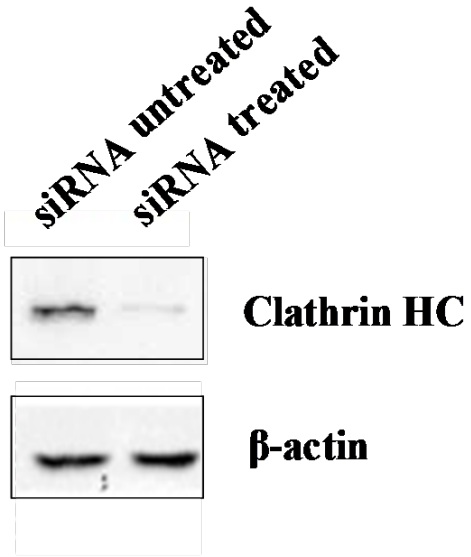


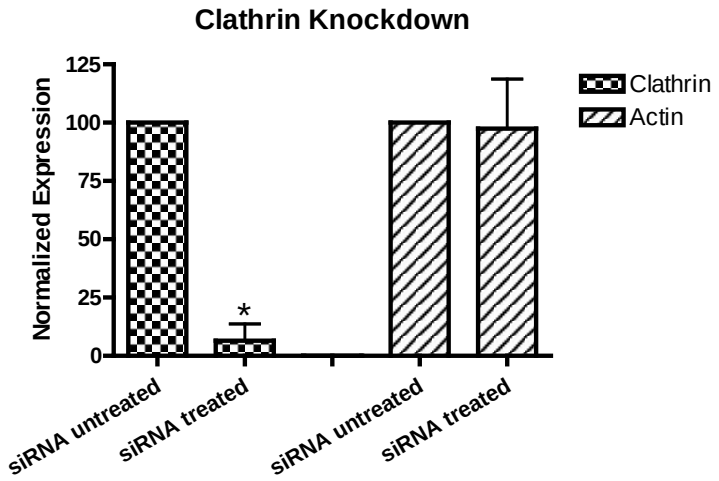
Chlorpromazine



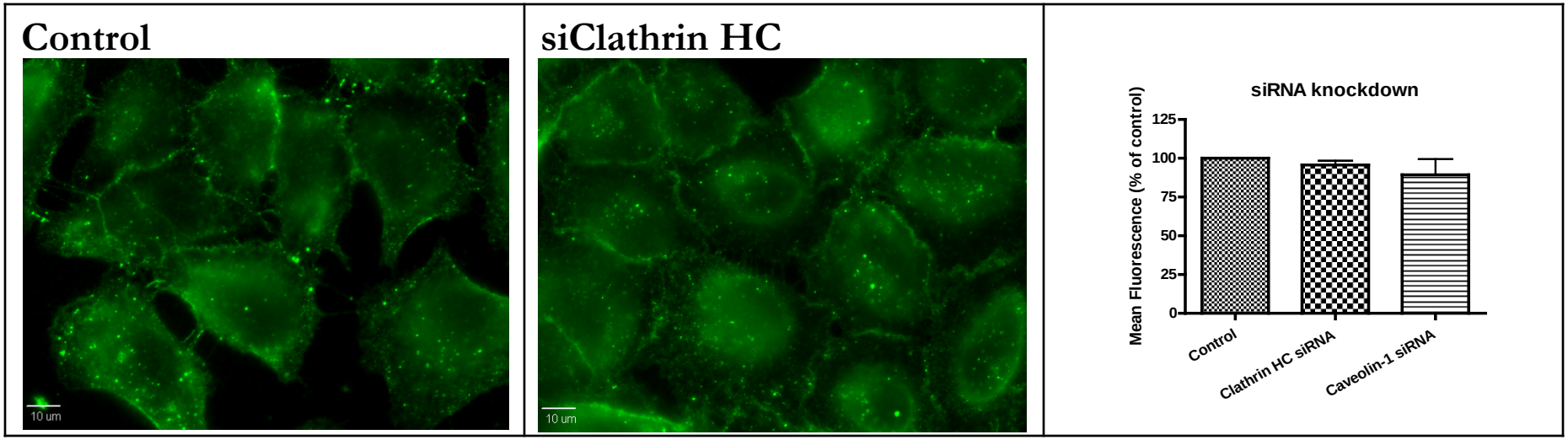


siRNA Knockdowns Clathrin

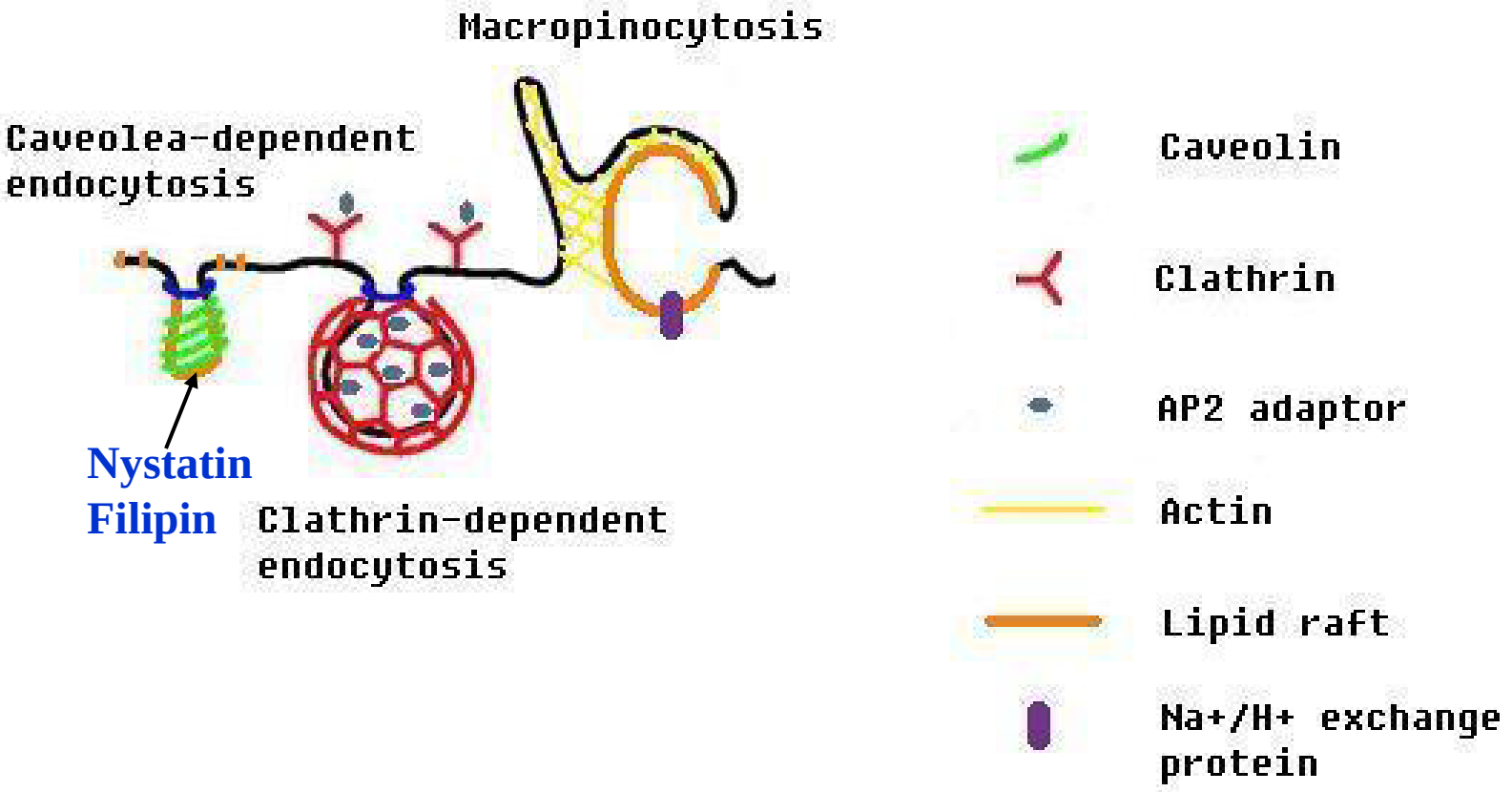




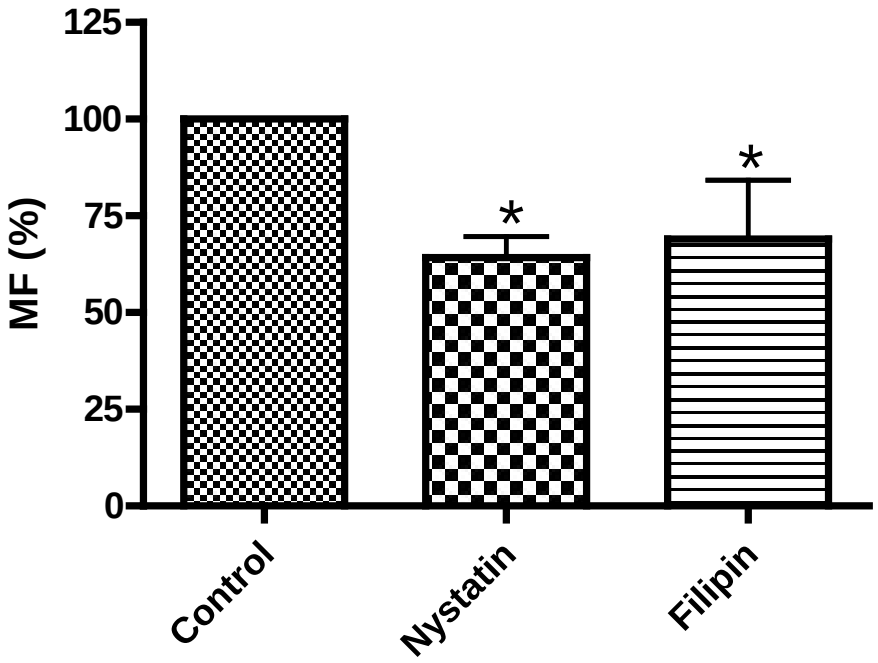
Unlikely Clathrin-dependent

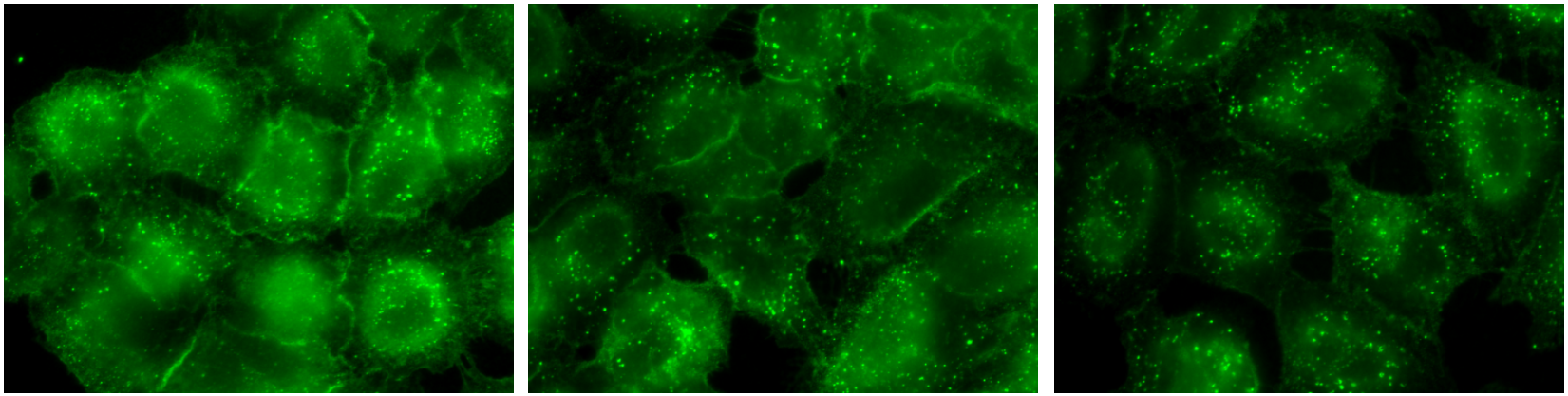


Lipid Raft, Caveolae Pathway

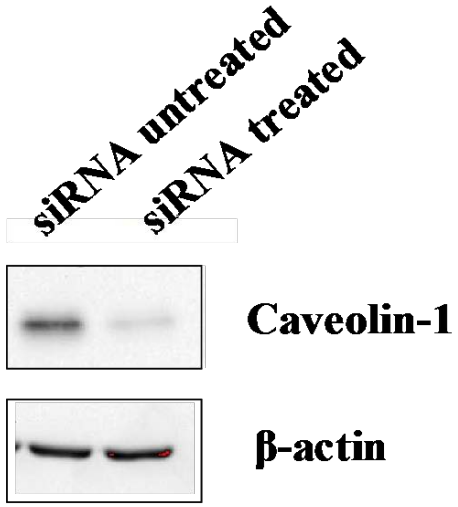


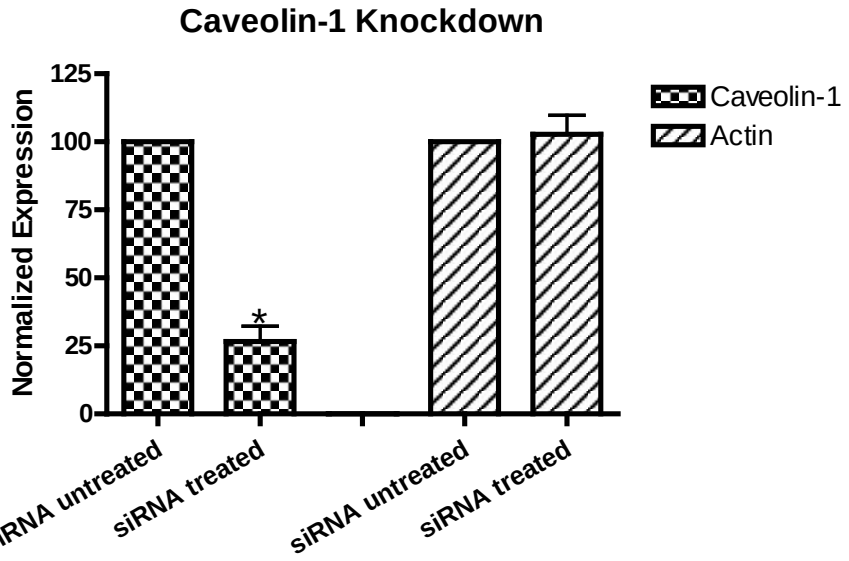
Lipid Raft / Caveolea Inhibition



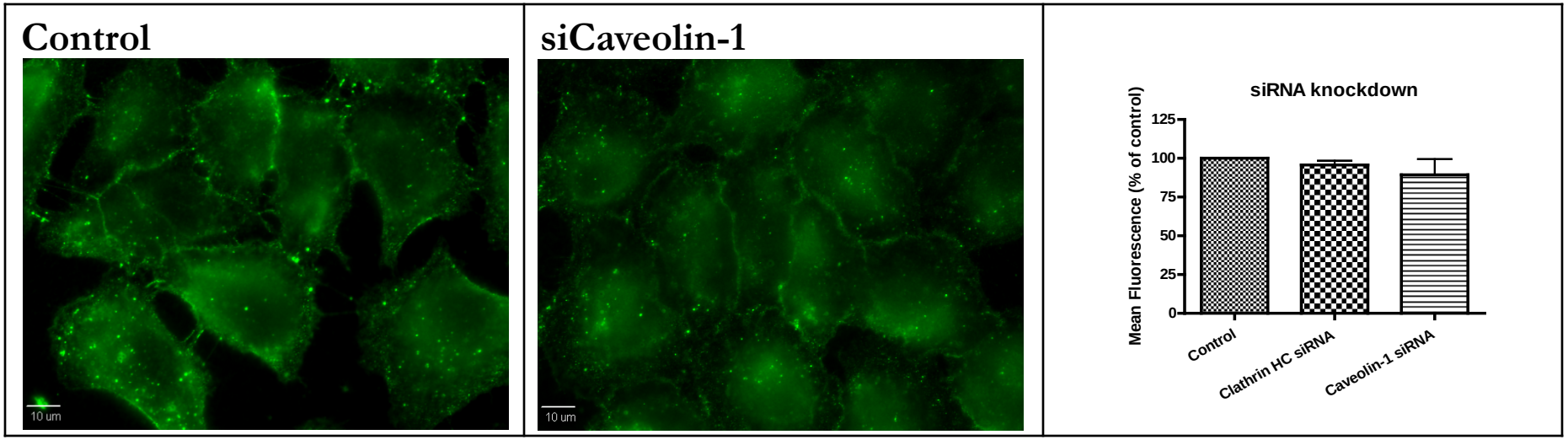


siRNA Knockdowns Caveolin-1

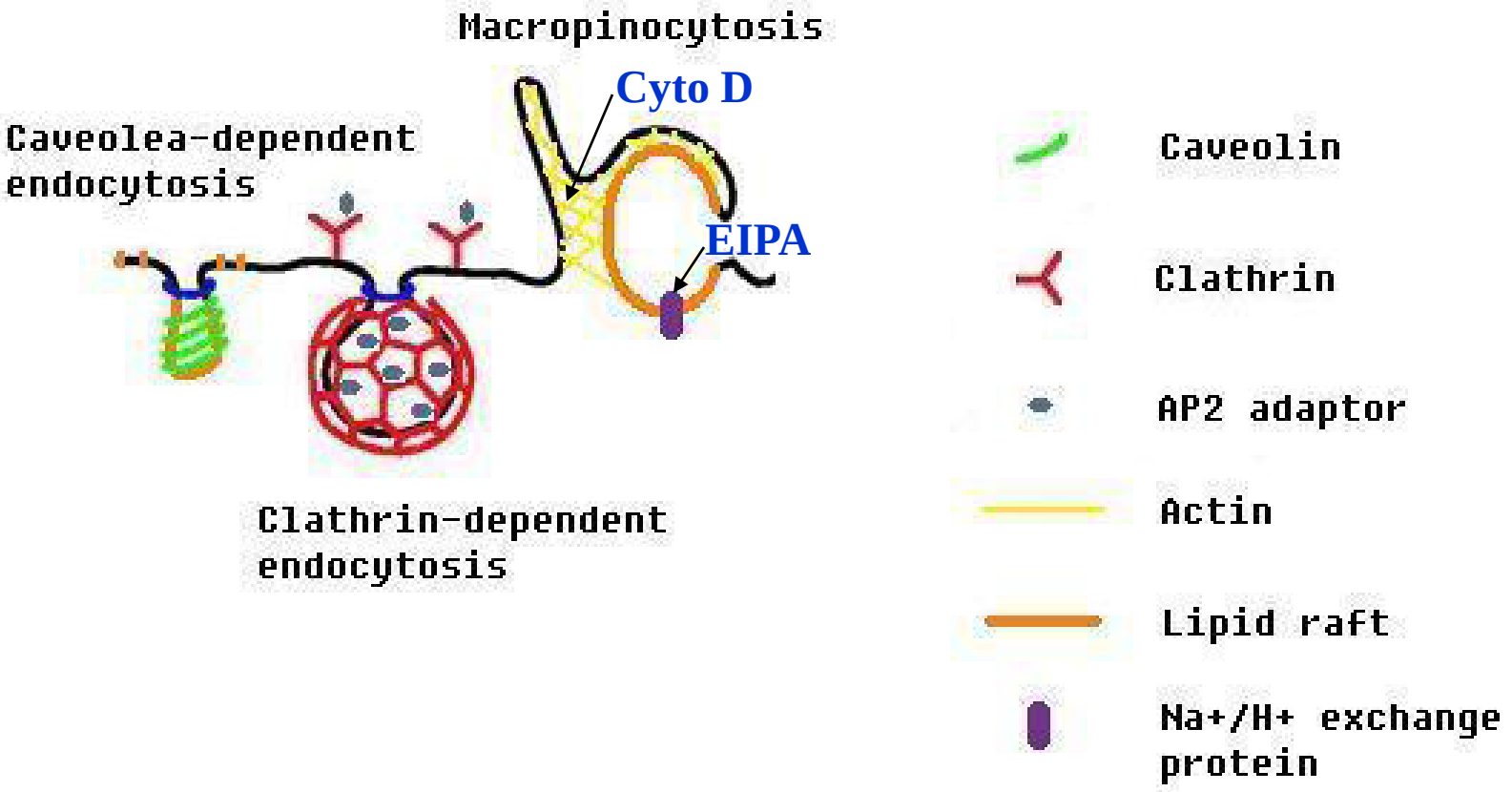




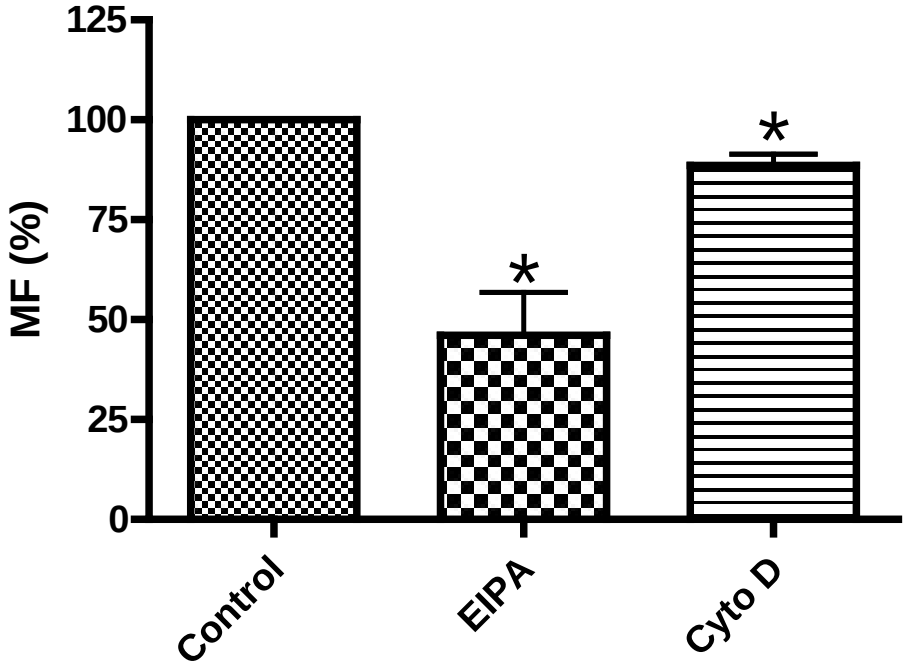
Lipid raft-dependent, but Unlikely Caveolin-dependent

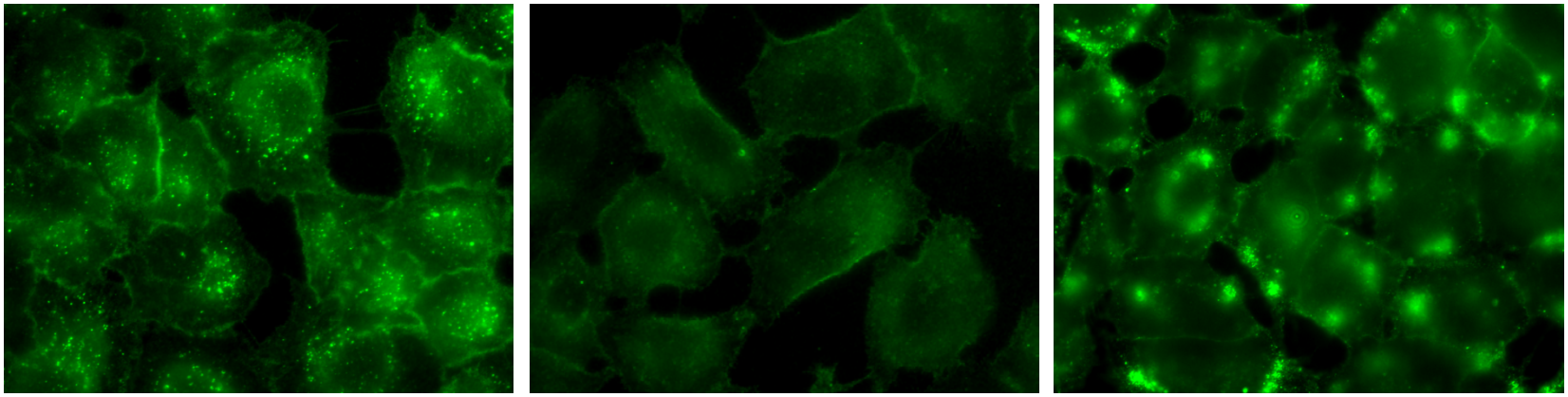


Macropinocytosis



Macropinocytosis Inhibition (≤ 5 μm, actin-dependent)

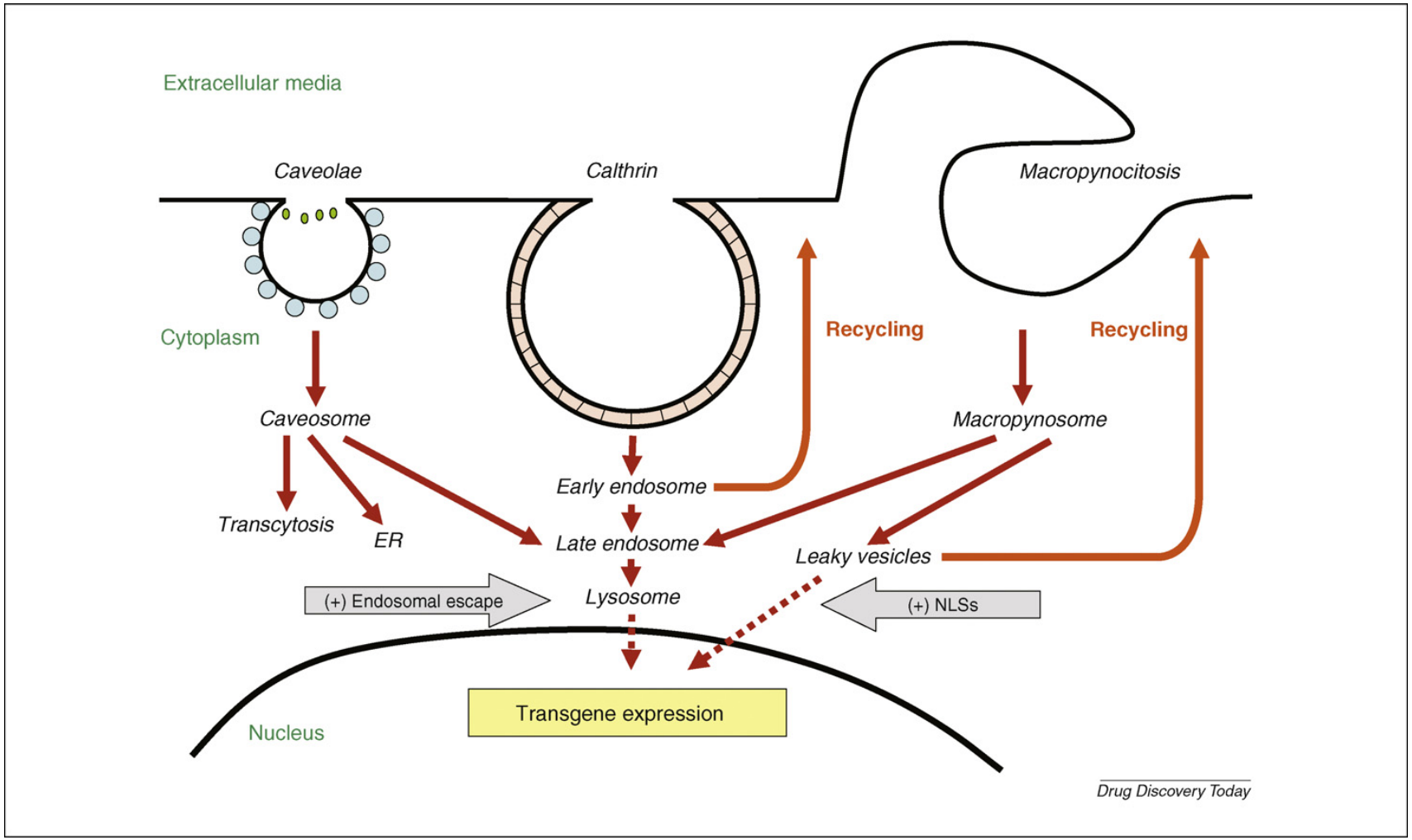




Inhibitors Are Not Quite Specific, Complementary Methods Are Needed

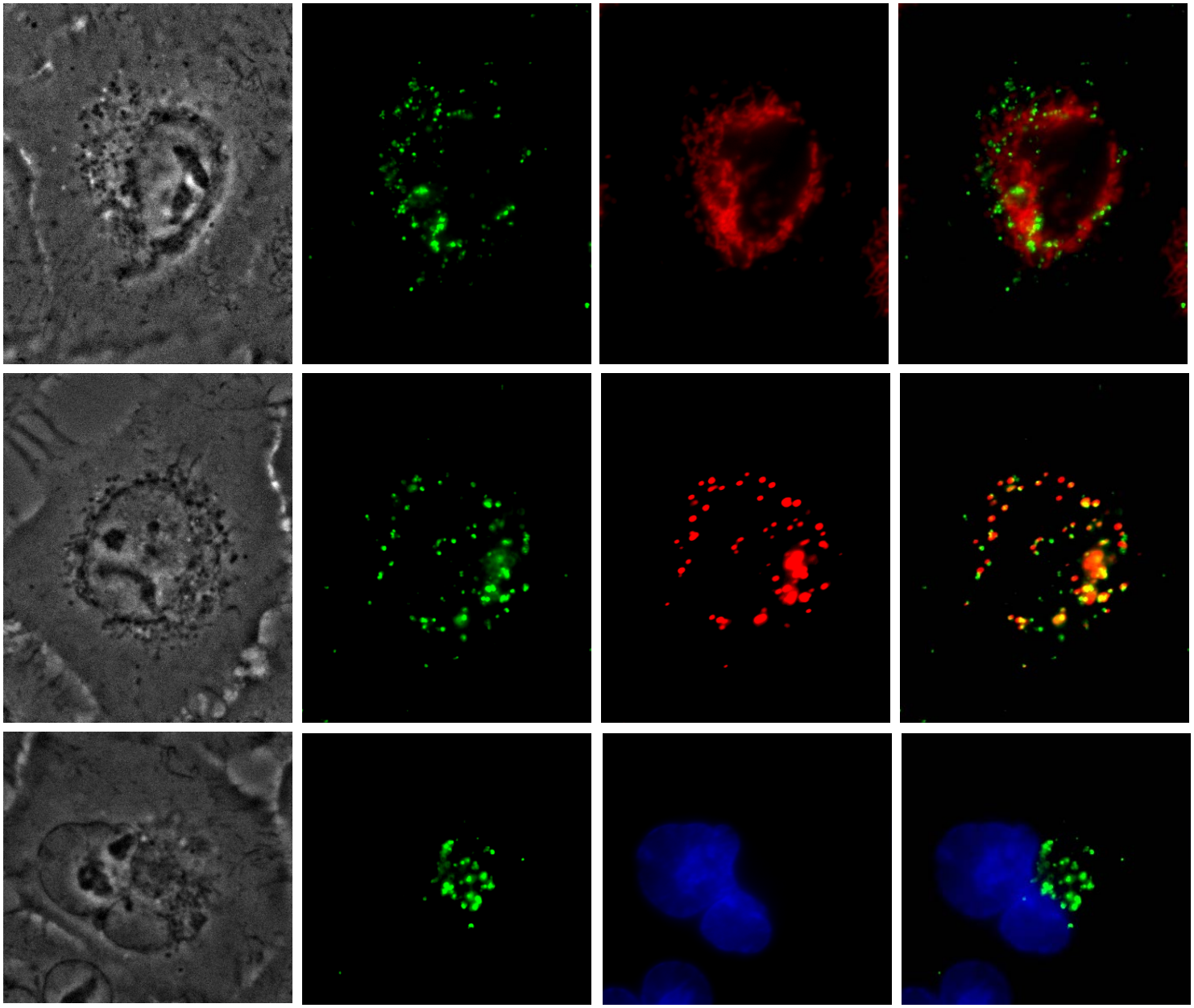
- Energy-dependent Pathway
  - $NaClO_3$ / NEM / Nigericin / Nocodazole / Sodium azide / Valinomycin
- Clathrin-dependent Pathway
  - CytoD / MβCD / NEM / Nocodazole
- Lipid raft, Caveolae-dependent Pathway
  - CytoD / Filipin / MβCD / NEM / Nystatin
- Macropinocytosis
  - CytoD / EIPA / MβCD

Destination & Fates of QD/R9 Complex



Drug Discovery Today, 13:1067-1074

Subcellular Localization

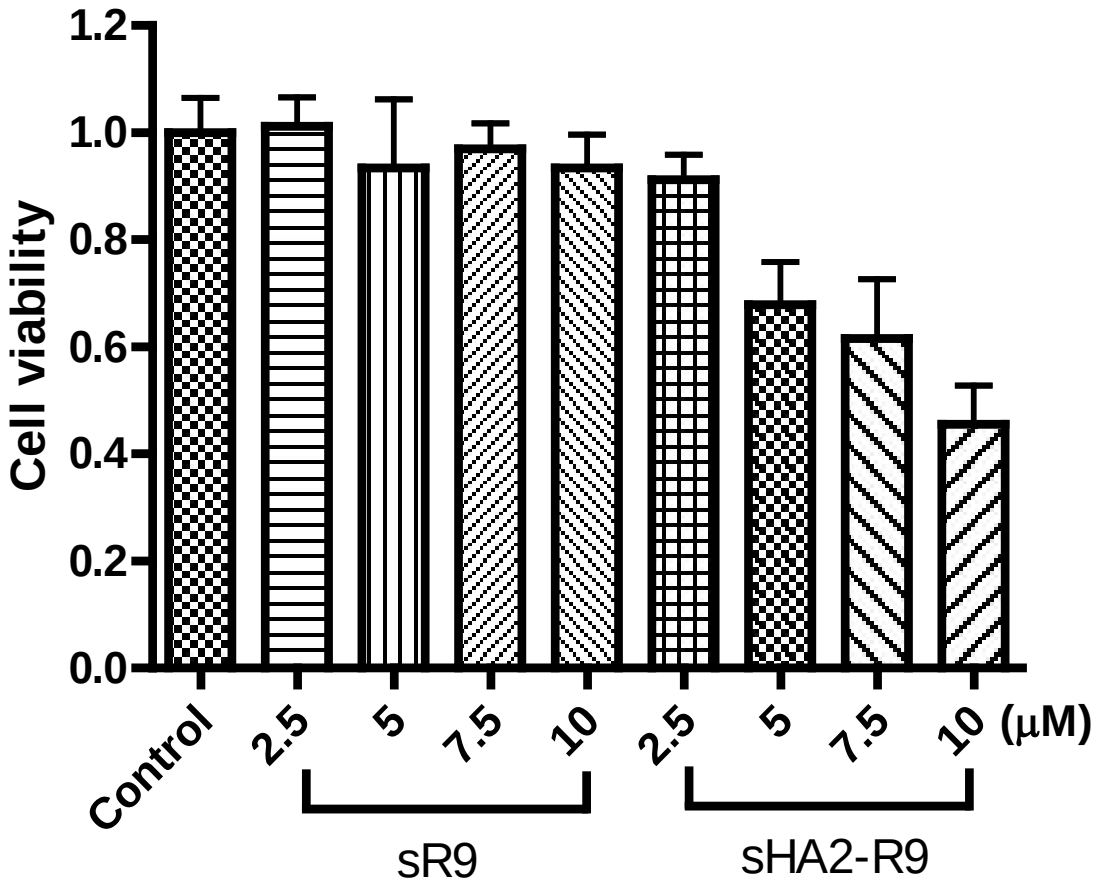


MitoTracker

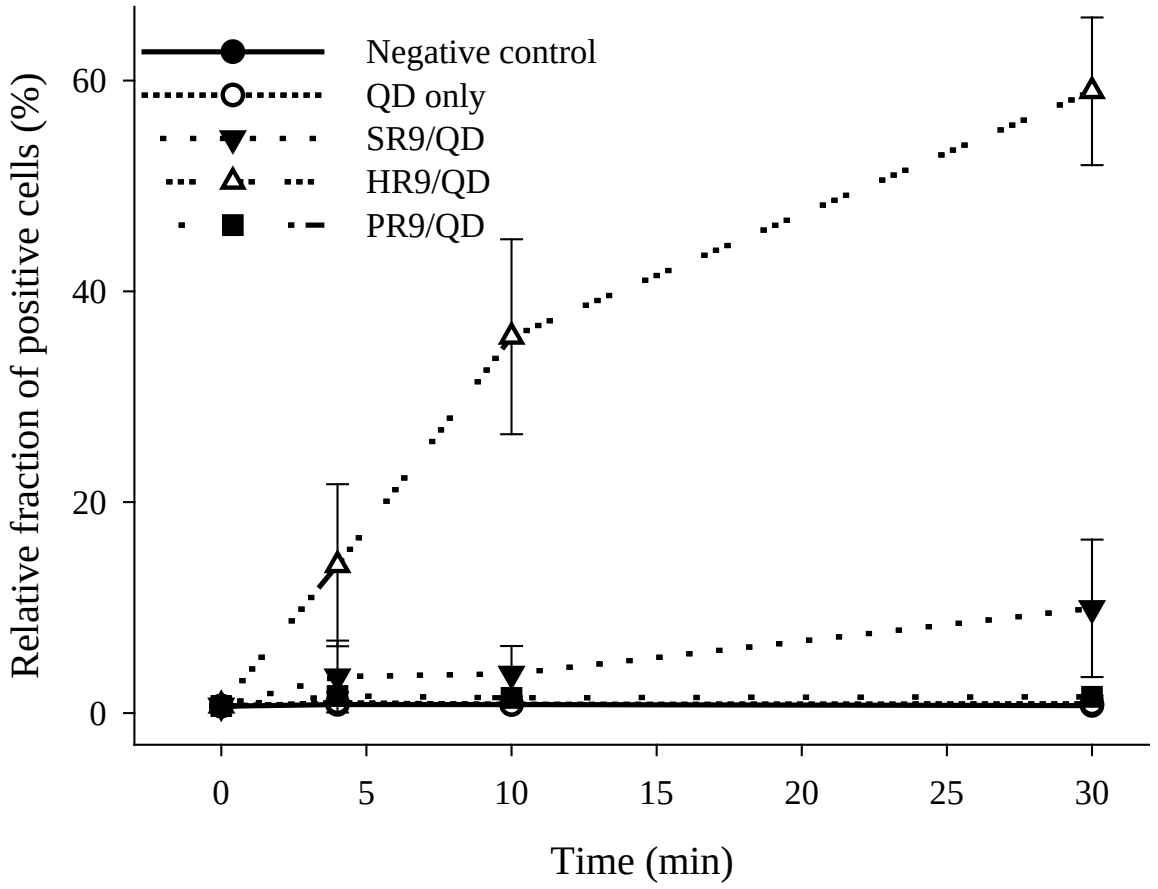
LysoTracker

Hoechst 33342

sHA2-R9 CPP is cytotoxic at effective conc. to deliver QDs

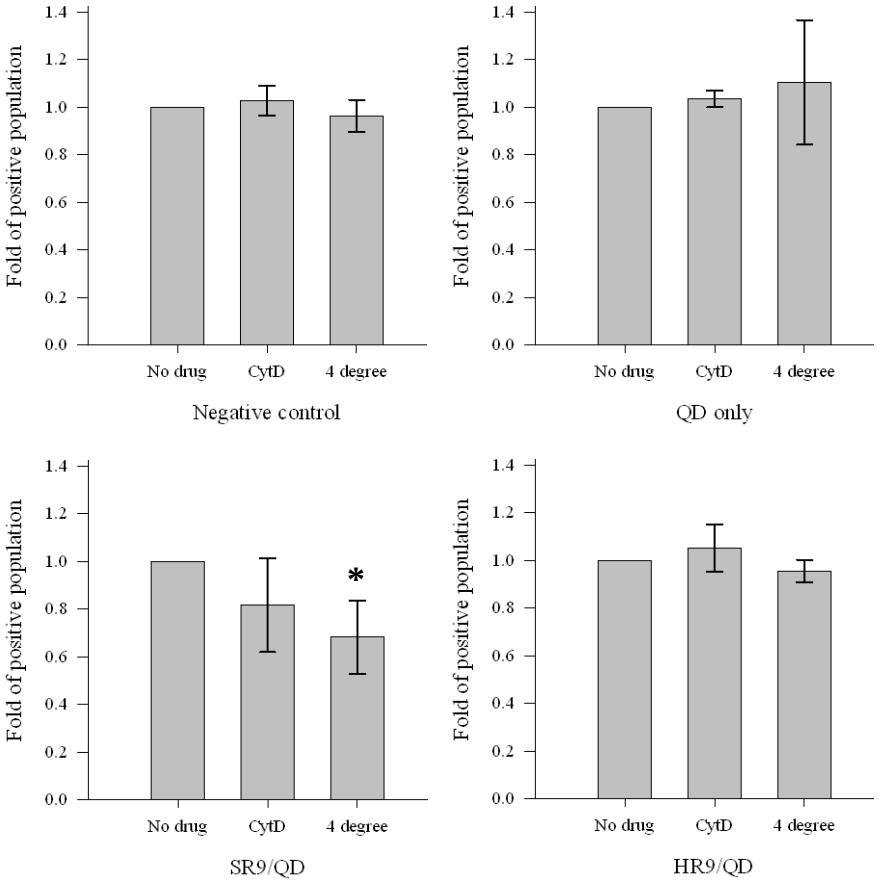


Alternative CPP – HR9
(Direct membrane penetration)



Liu, et al. 2011. Biomaterials, 32:3520-3537

HR9 Uses Direct Membrane Translocation



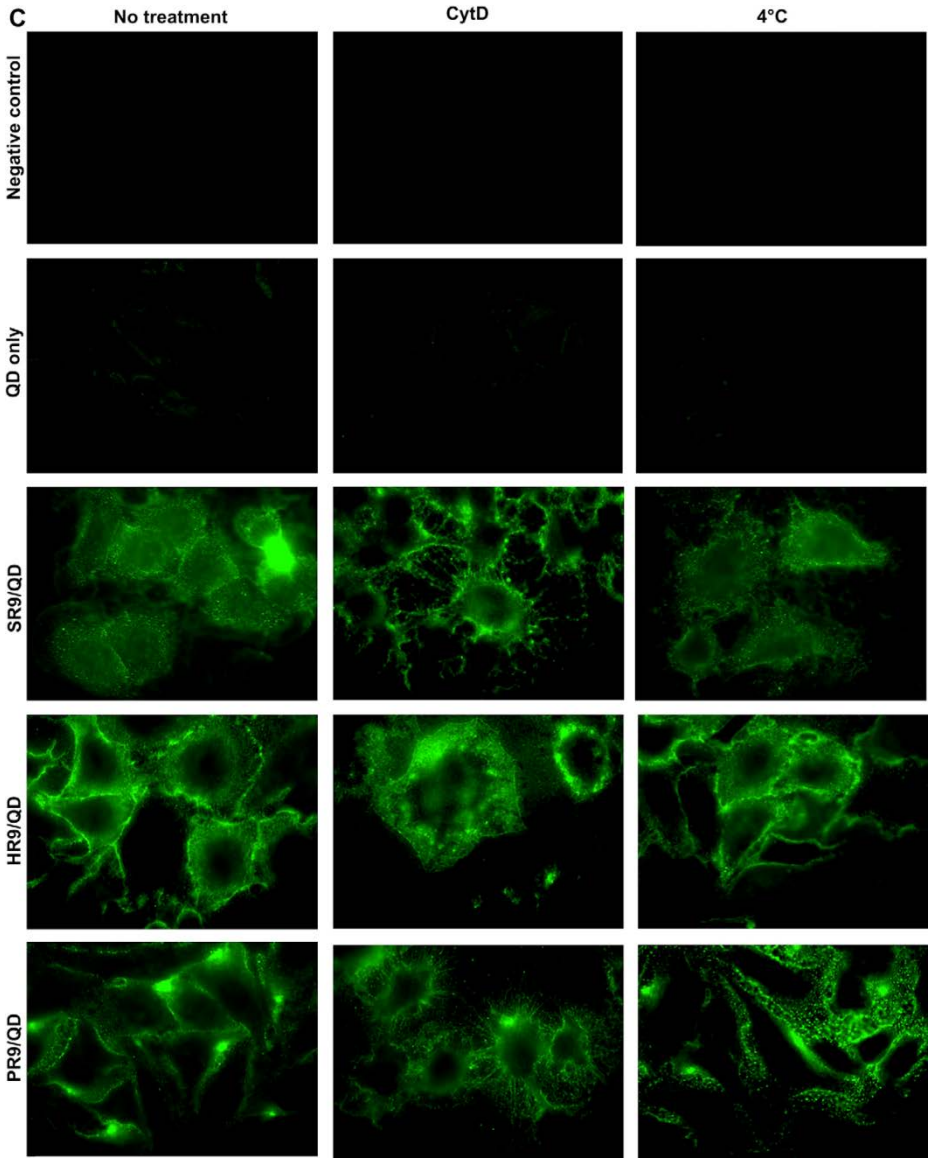
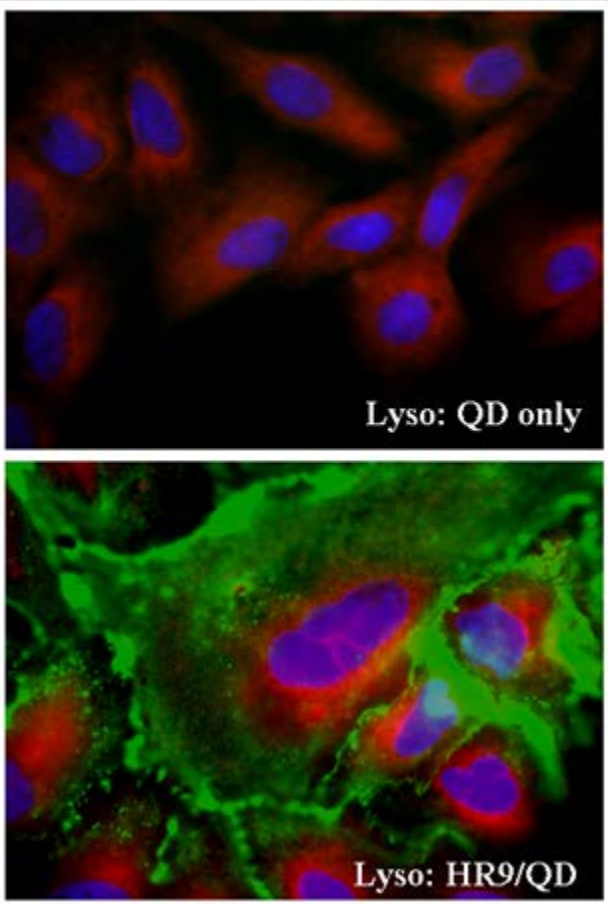


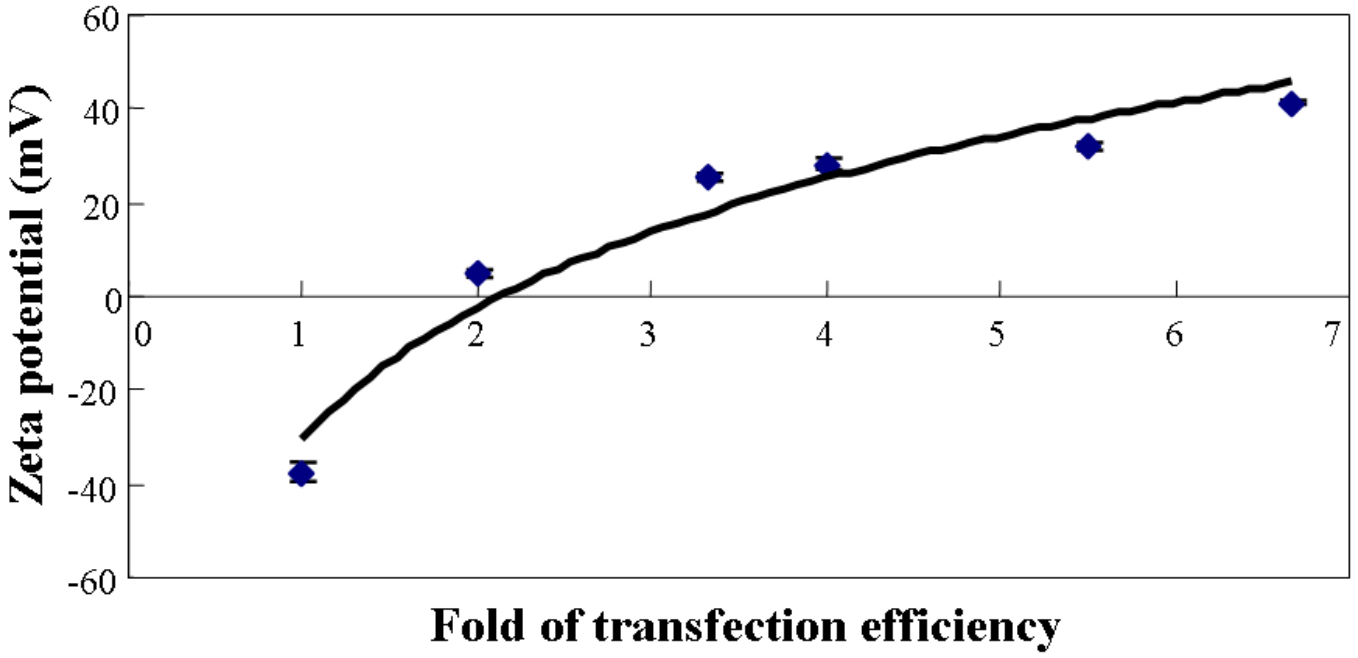
Fig. 12. Effects of endocytic inhibitors on membrane translocation of CPP/QD complexes. Cells were treated with or without CytD or 4^{o}C followed by CPPs/QD treatment for 4 min (26).

HR9/QD not associated with lysosome



Liu, et al. 2011. Biomaterials, 32:3520-3537

Zeta Potential and Transduction Efficiency



Conclusions

- CPP-mediated nanomaterials (NM) enter cells via multiple pathways
- Internalization of CPP-NM is CPP composition dependent
- Pharmacological inhibitors need to be used in tandem with siRNAs
- Zeta potential may be a good predictor of transduction efficiency

Acknowledgement

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  - Dr. Jeffrey G. Winiarz (Missouri S&T Chemistry)
  - Dr. Katie B. Shannon (Missouri S&T Biological Sciences)

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  - Betty Liu (National Dong Hwa U.)
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