QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Stealth particles for targeted delivery of drugs to brain tumors

Trudy M. Forte, PhD

June 27, 2007

Gliomas

Brain tumors arising from glial (neuroeptithelial) cells.

Glial cells come in several flavors: astrocytes, oligodendroglial and ependymal cells.

Gliomas show aggressive growth.

Grade IV gliomas are referred to as glioblastoma multiforme (GBM) and have multiple genetic and chromosomal abnormalities.

Glioblastoma multiforme (GBM) Therapies

Surgery

Radiation

Chemotherapy

Result: 1 year average survival time

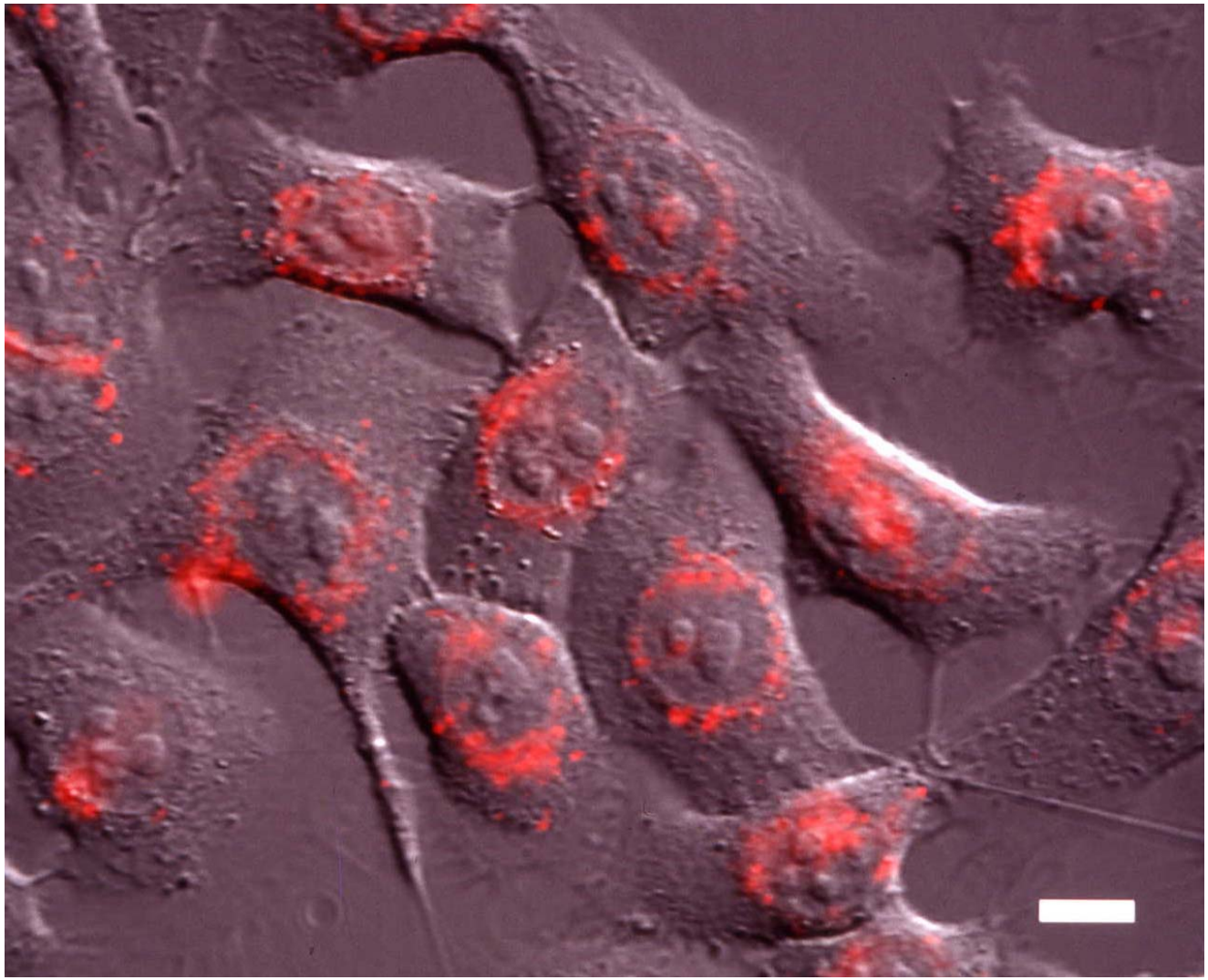
Boron Neutron Capture Therapy (BNCT) for Brain Tumors

Principal: neutron beam used to bombard Boron atoms delivered to tumor cells. High energy particles generated cause oxidation events in cells that destroy tumor cell.

Assumes one is able to get Boron into cells in high concentration.

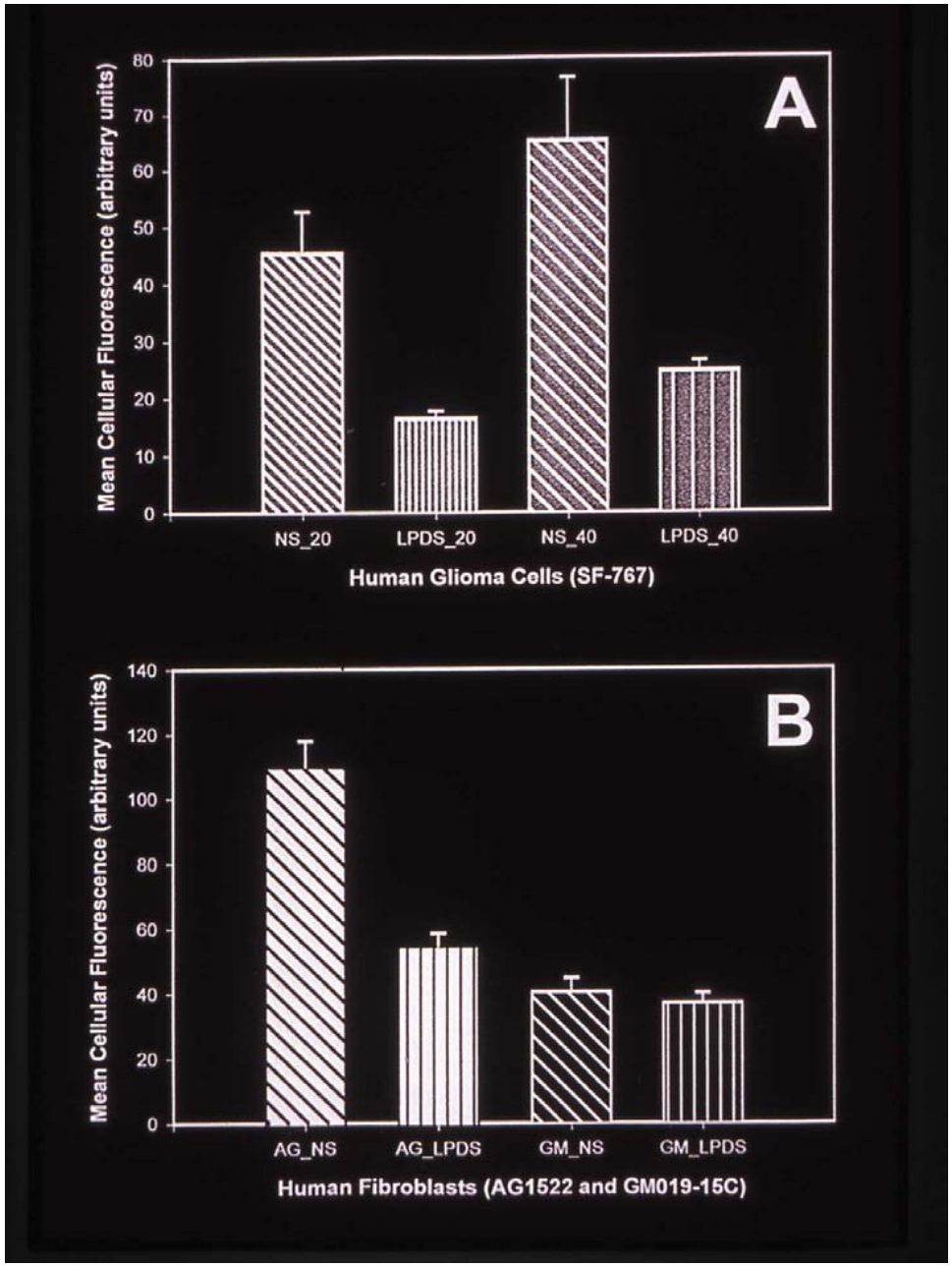
Boronated protoporphyrin (BOPP) was used as the therapeutic agent to target cells.

SF-767 cells: Distribution of boronated porphyrin



Callahan et al, *Int J Rad Biol*, 1999

Uptake of BOPP requires LDL and the LDL receptor

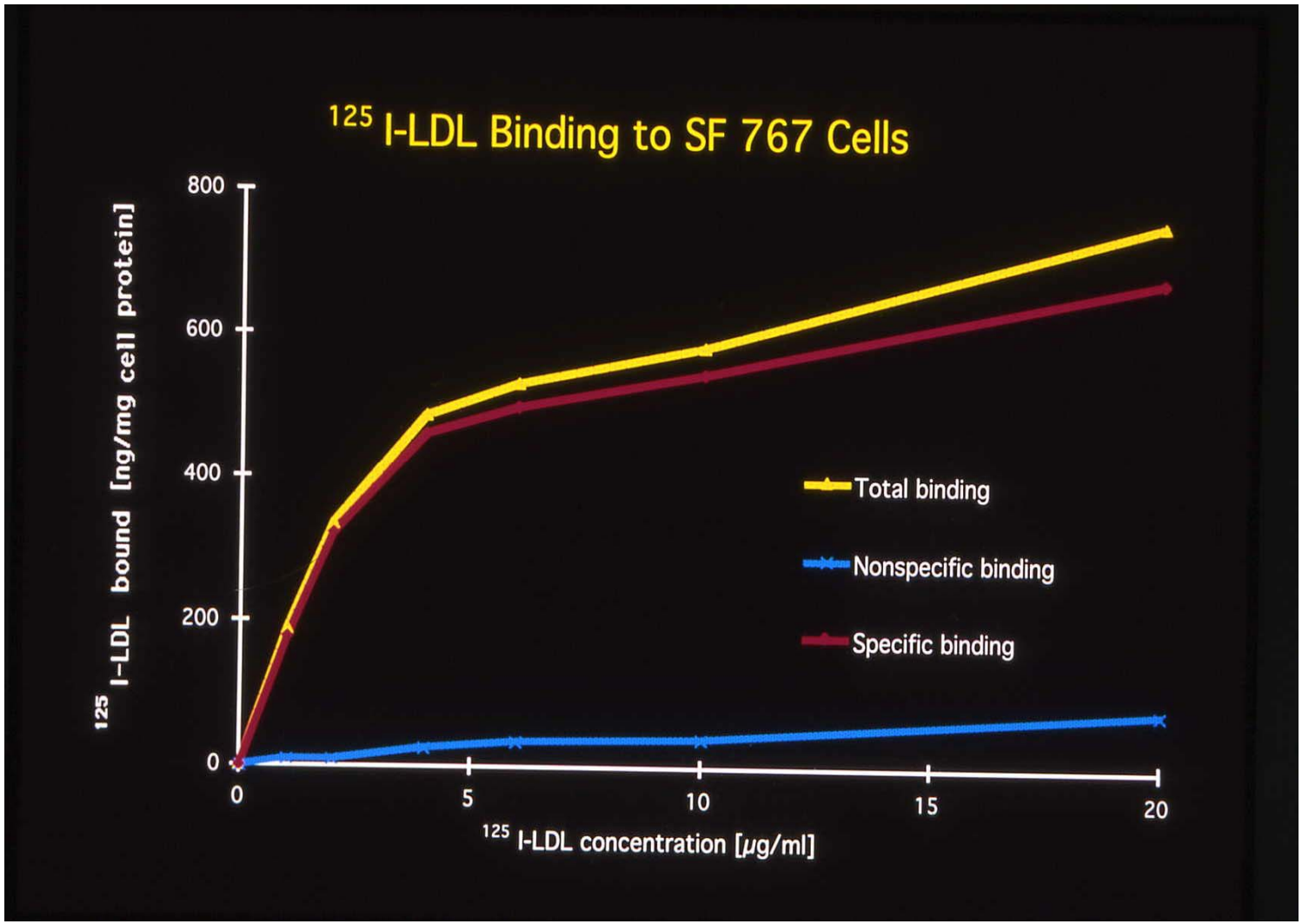


SF 767 cells ± plasma lipids

Normal vs LDLR defective cells

Callahan et al, *Int J Rad Biol*, 1999

Kinetics of LDL Binding to SF 767 Cells



Maletinska et al, *Can Res* 2002

LDL Receptors on GBM Cell Lines

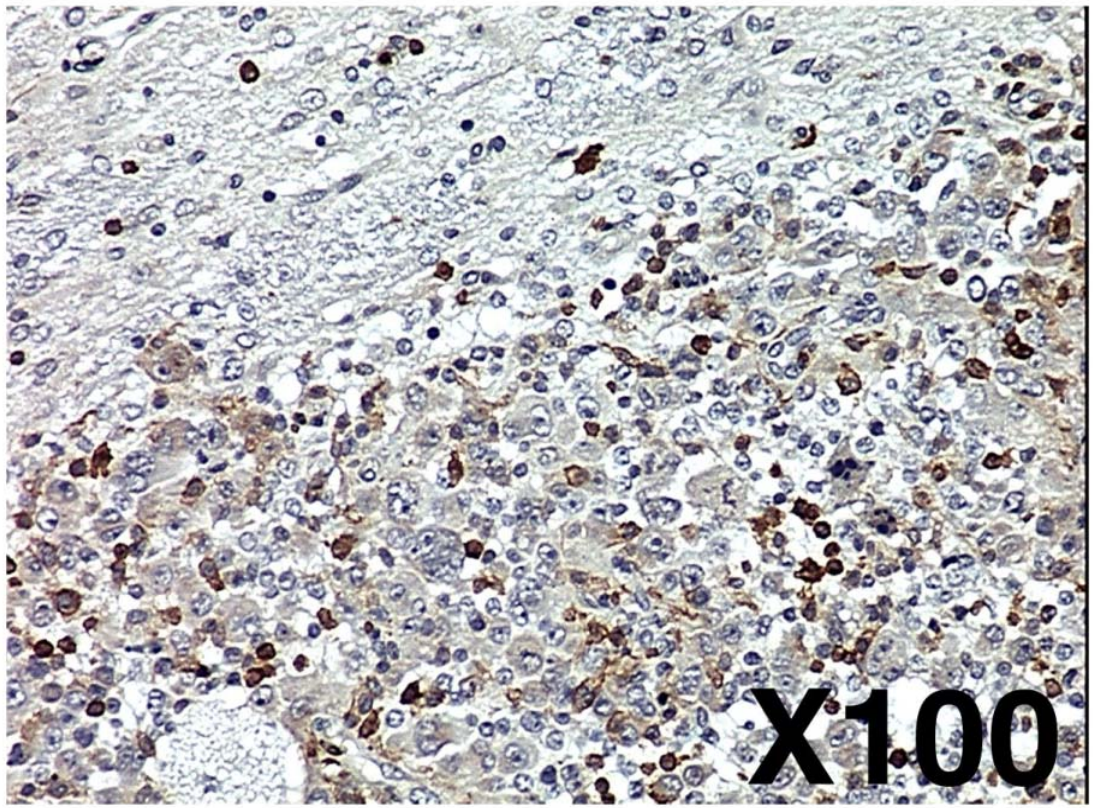
Brain Tumor Cell Line	LDL Receptors per cell
SF-767	288,000
SF-763	950,000
A-172	923,000
U-251	128,000
U-453	311,000
SF-539	252,000

Maletínská et al, Cancer Research, 2000

Xenograft: Human U251 GBM cells implanted into athymic rat

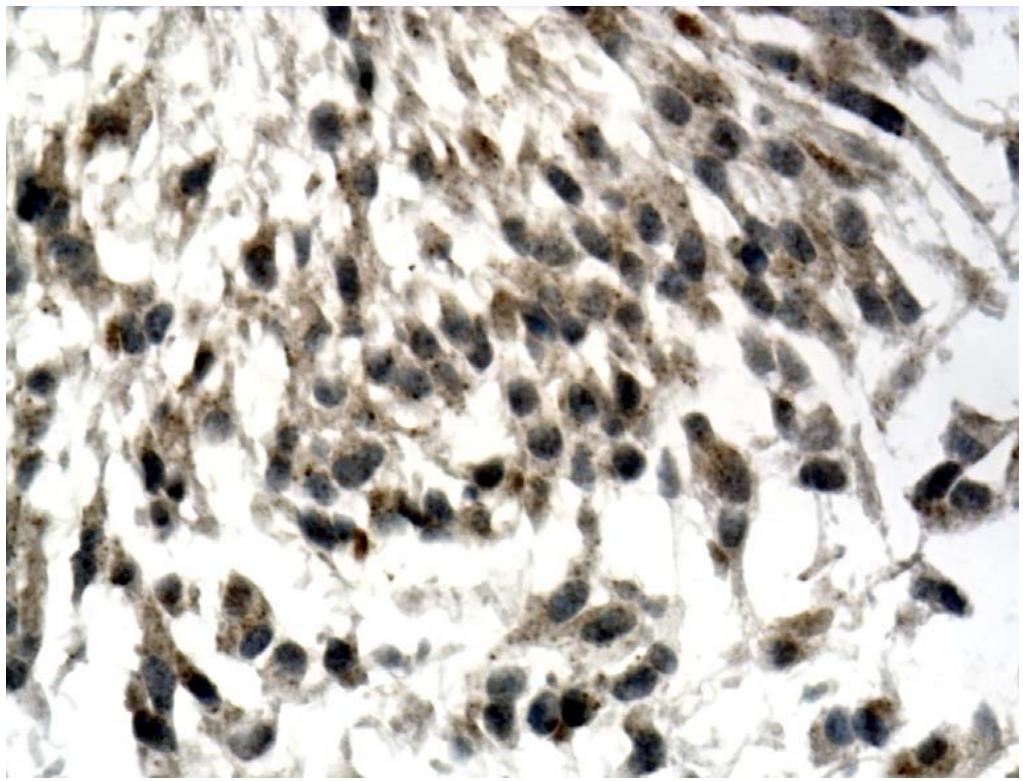
Anti-LDL receptor antibody used to localize LDLR

Normal rat brain →



← Tumor

Human GBM Biopsy Sample Demonstrates LDLR



71% of tumors sampled had high expression of the LDLR

CONCLUSIONS

- GBM cells are distinguished by the upregulation of LDL receptors (LDLR).

  Why is this observation important?

  Neurons and normal brain cells have few LDLRs.

- The LDLR is potentially a molecular target for the delivery of anti-cancer agents to the tumor.

- It is possible to use LDL or synthetic LDL to target therapeutics to the GBM cells.

LDL as Drug Delivery Vehicle

- Problems with using native LDL as drug delivery vehicle:
  - LDL is easily oxidized
  - Difficult to isolate in large quantity
  - Requires source of fresh plasma (possible disease transmission potential)
  - Has variable composition and size

Solution to Problem

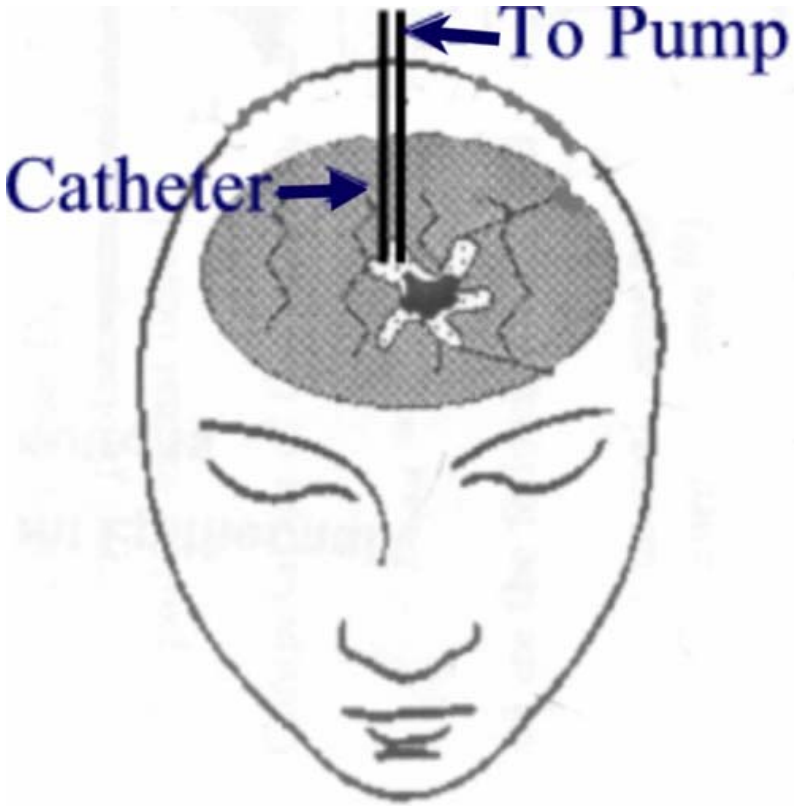
QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Drug
Payload

Design a Trojan Horse (synthetic LDL) to deliver anti-tumor drugs to GBM via the LDLR

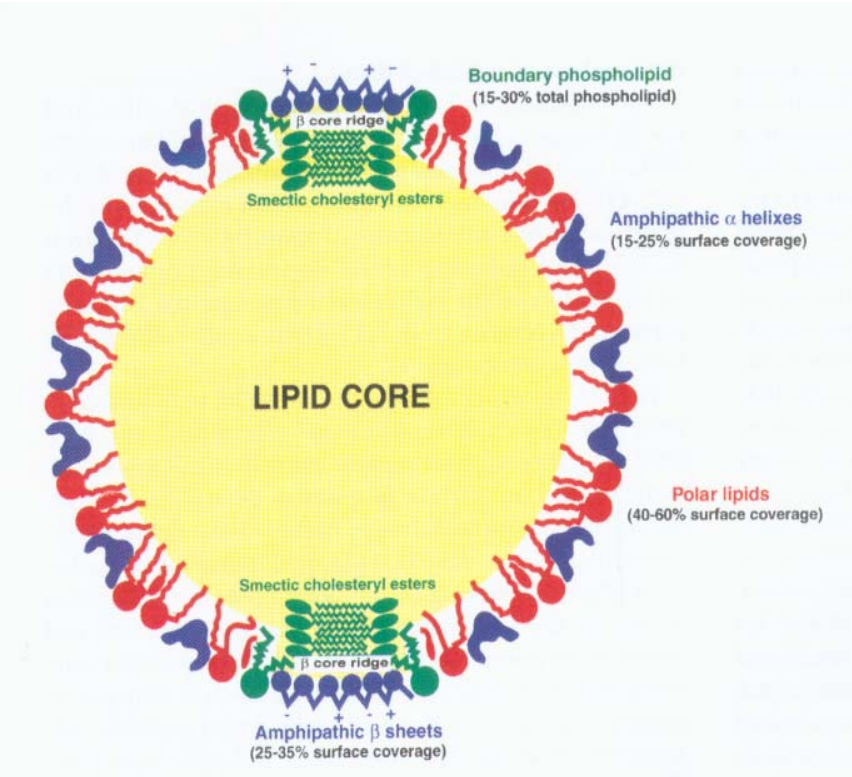
Spare the neurons; toxic drug not delivered to healthy cells

Drug Delivery to the Brain Tumor



- Direct injection into tumor using Convection Enhanced Delivery (CED)
- Less invasive than conventional surgery or chemotherapy

LDL and apoB Protein

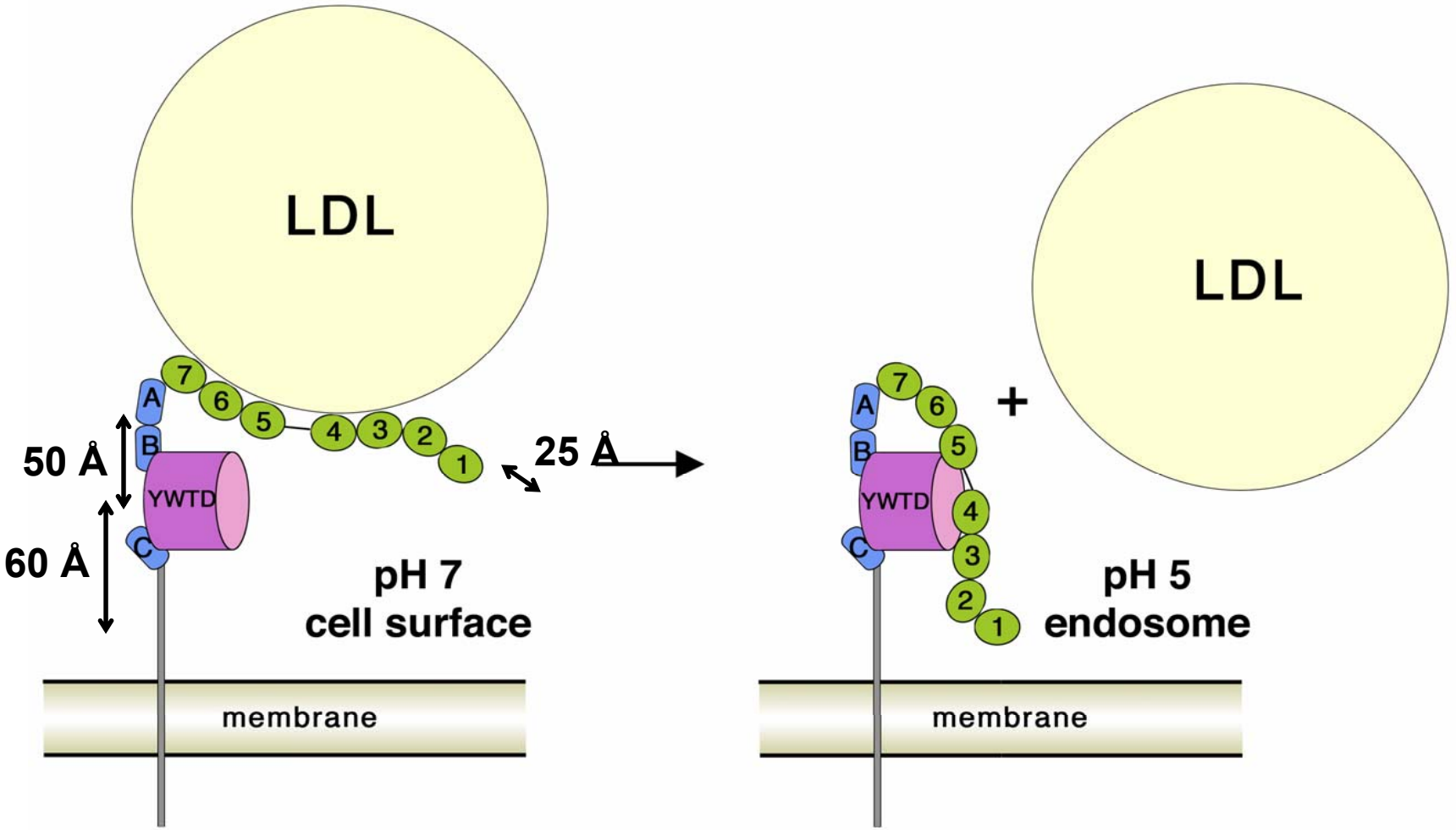


24 - 27 nm

From J. Segrest, *JLR*, 2001

- Cholesterol from liver transported in LDL
- Phospholipid shell
- apoB protein surrounds LDL
- a.a. 3359-3367 of apoB is the ligand for LDL receptor

Model for LDL binding to LDLR and intracellular release of LDL

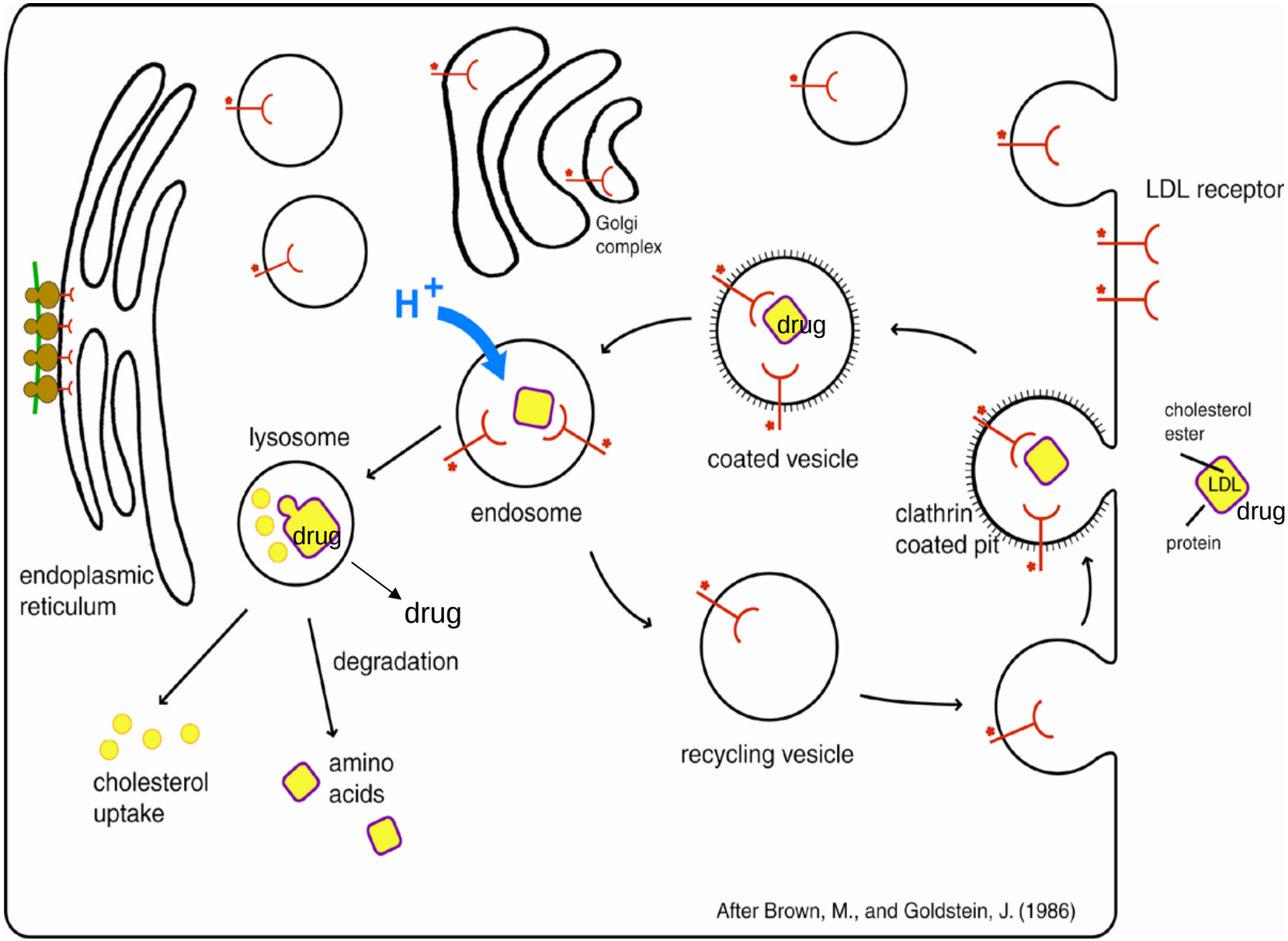


pH 7: binding competent

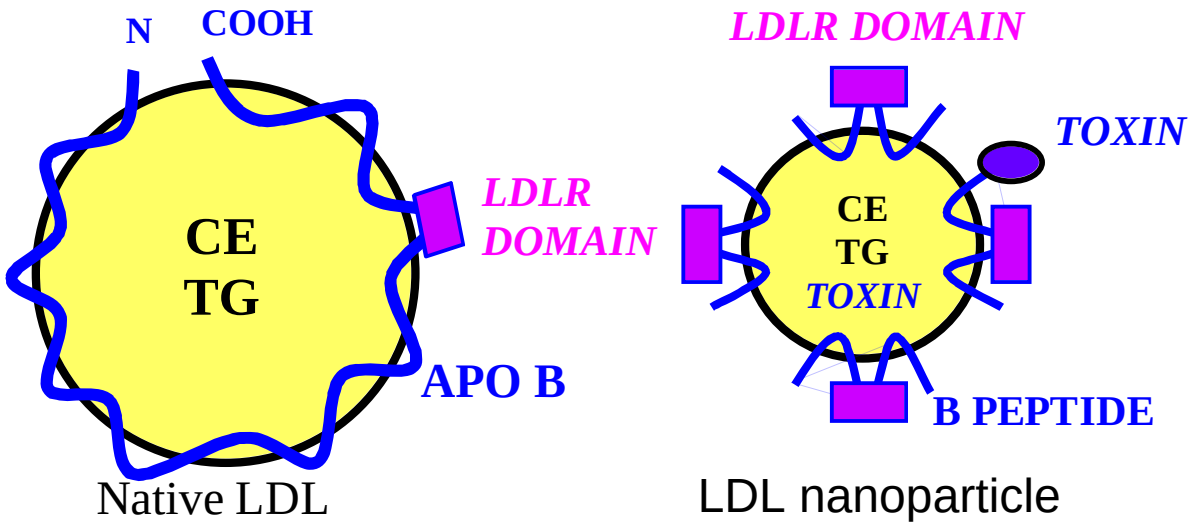
pH 5: inhibition of ligand binding by intramolecular contacts between the propeller and the ligand-binding modules

From S. Blacklow

The LDL receptor transports cholesterol-carrying lipoproteins into cells



Design Principal of a synthetic nano LDL for targeting GBM cells



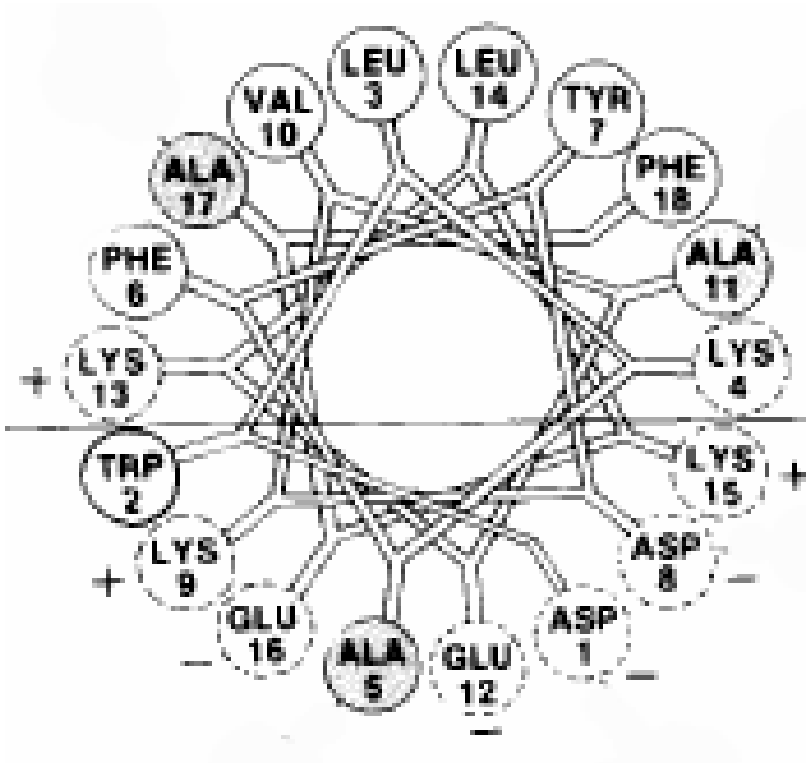
Peptides rather than full length apoB used since apoB is difficult to purify.

Using peptide technology, nano LDL can be made reproducibly in large batches.

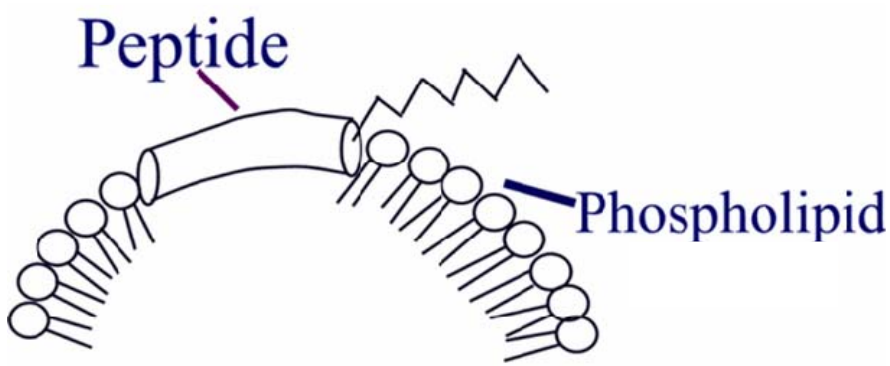
Lipophilic anti-cancer drugs can be transported in lipid core.

Synthetic Peptide

DWLKAFYDKVAEKLKEAFRLTRKRGLKLA



Anantharamaiah, 1985

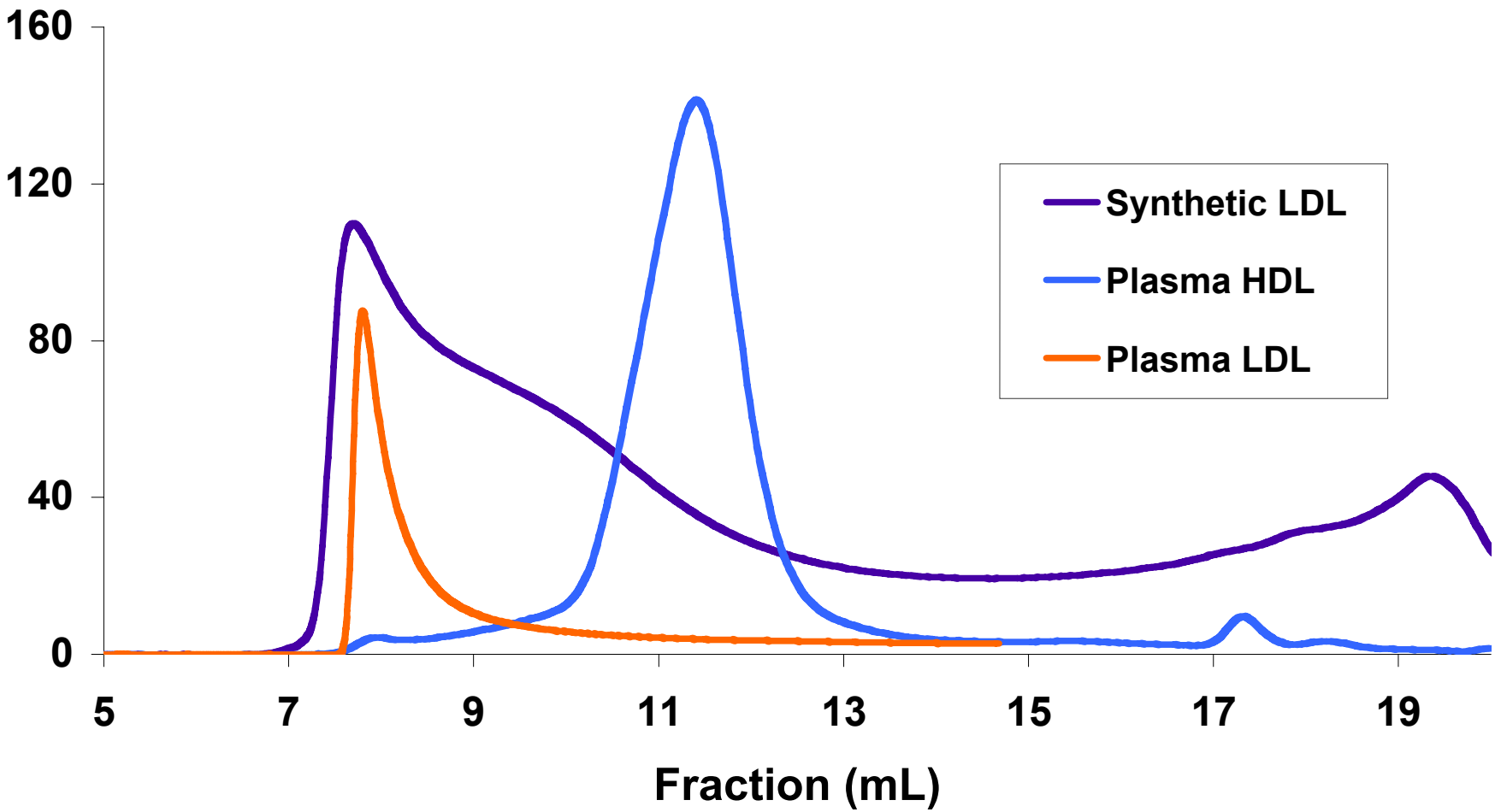


Construction of NanoLDL

- Combine PC, TG, and CE in Tris-saline buffer
- Sonicate for 1 hour on ice
- Extrude emulsions using 0.03 µm filter
- Add peptide and dialyze
- Peptide capped with N-terminal acetyl group and C-terminal amide group
- Recovery: 78%

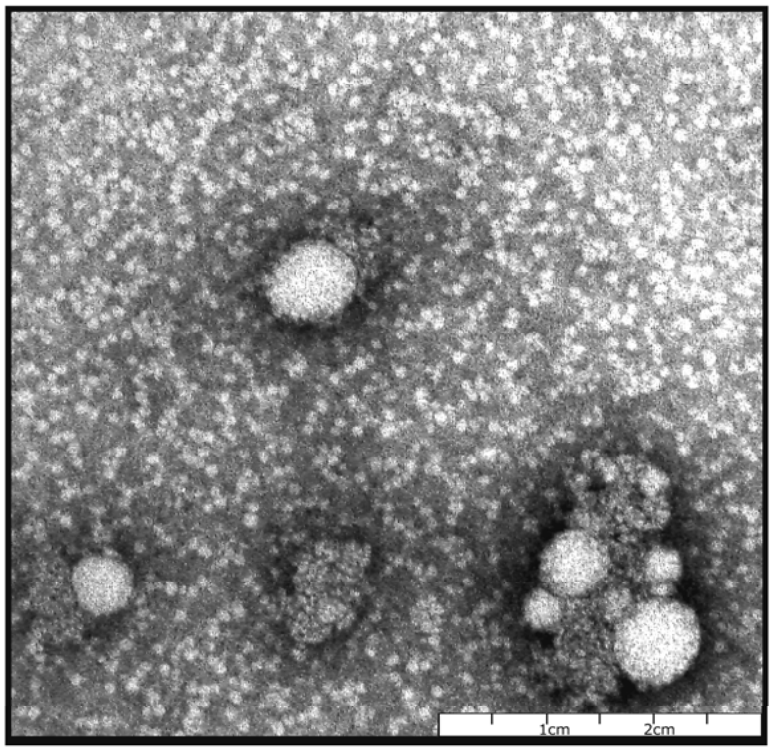
[adapted from Baillie et al. JLR, 2002]

Size of Synthetic LDL (FPLC)

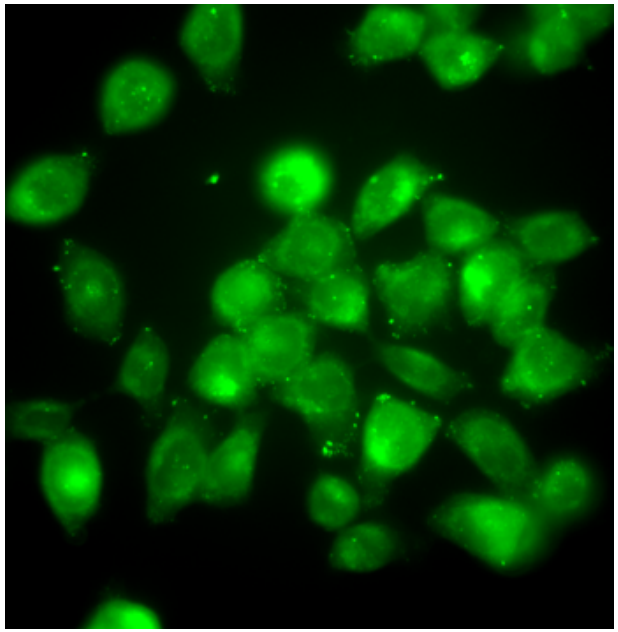


EM of nanoLDL isolated at d1.063-1.21 g/ml

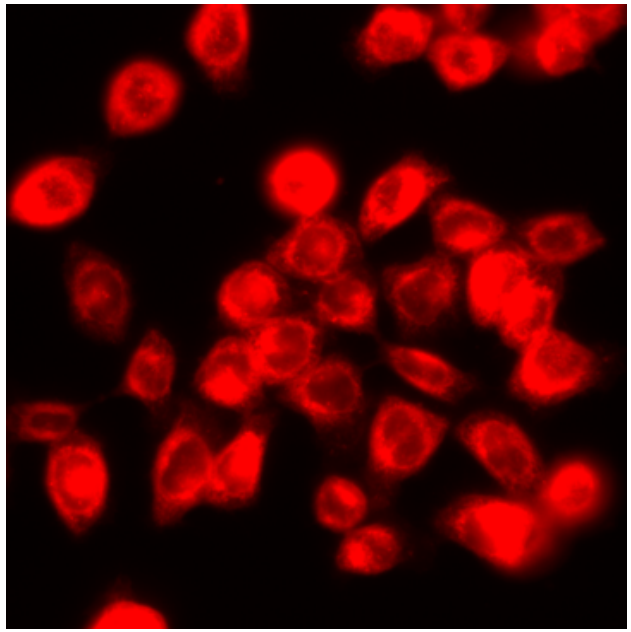
(~10 nm average size of particles)



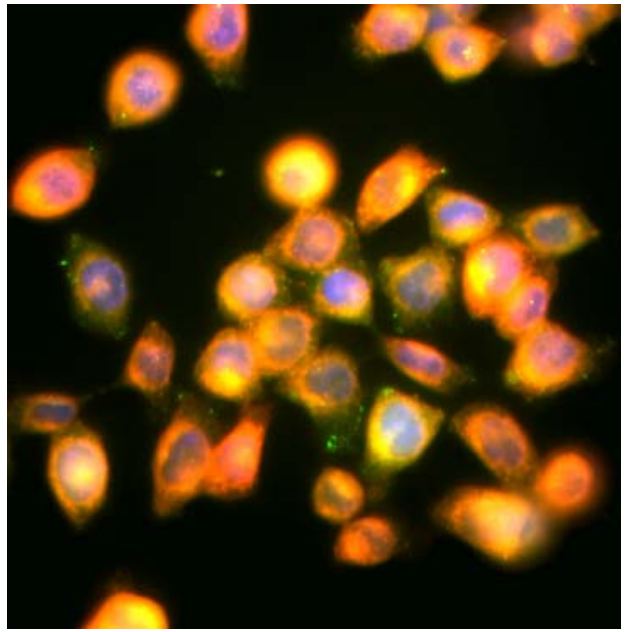
Synthetic LDL Uptake



FITC Labeled Peptide



DiI Labeled Lipids



Merged Image

SF-767 Tumor Cell Line, fixed cells

15 µM peptide, 6 hr, 37°C

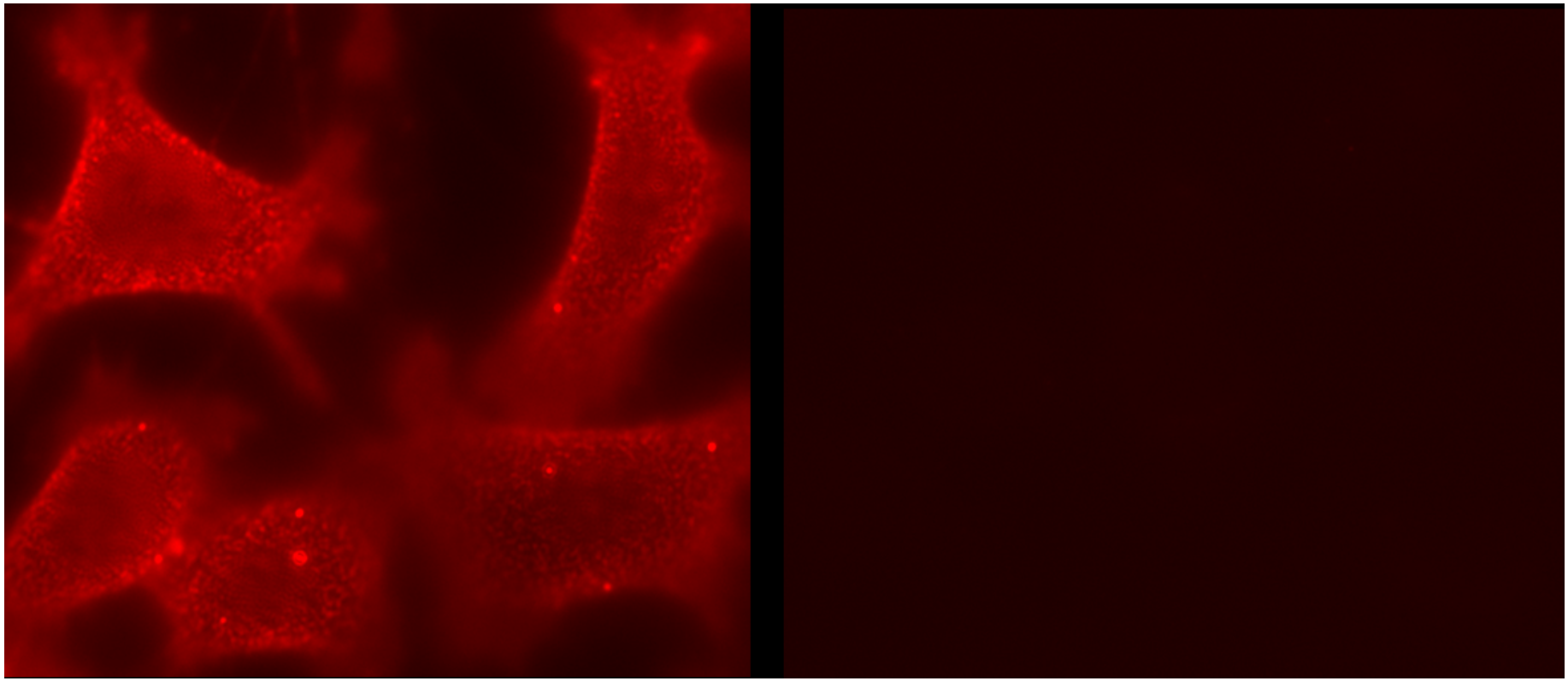
Uptake of nanoLDL into living SF-767 cells

1.5 μM peptide, 1 hr, 37°C

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

FITC	DiI	Merged	Lysotracker
peptide	lipid		lysosome

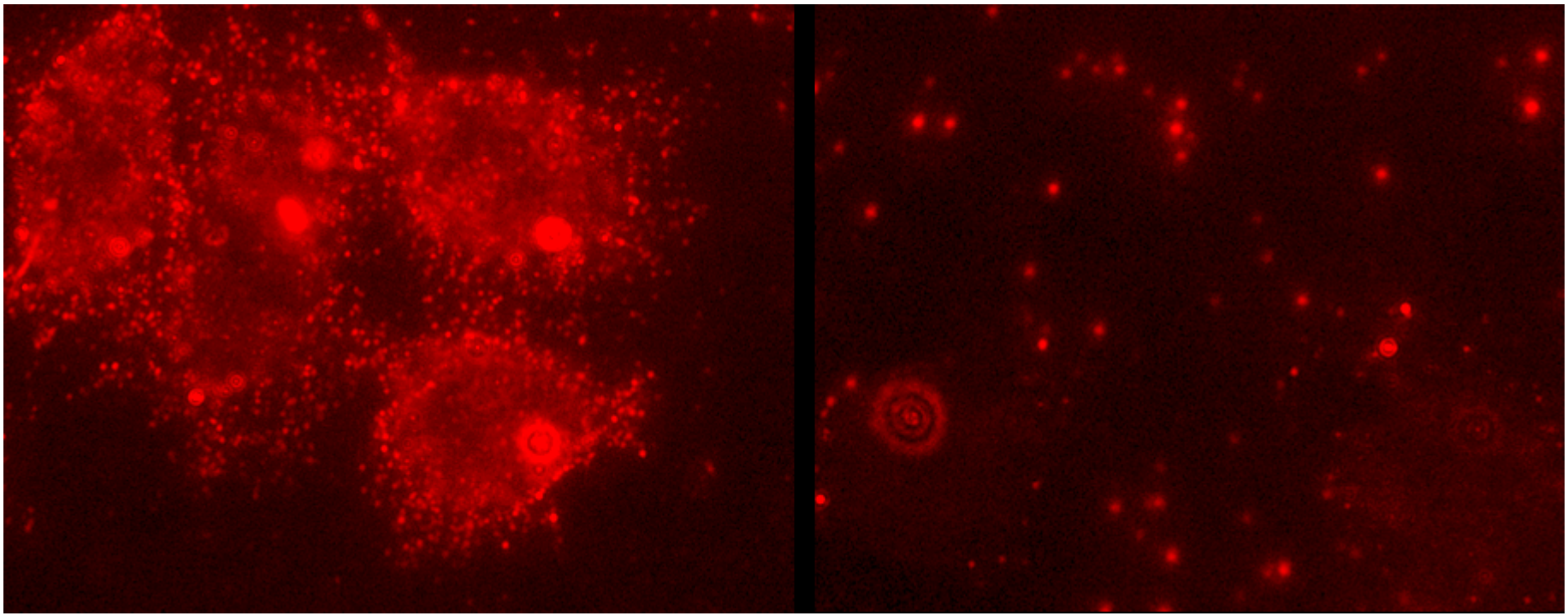
Inhibition of LDLR-specific Binding: Suramin



sLDL-DiI **sLDL-DiI + Suramin (10mM)**

SF-767: 1hr, 4°C

Specific LDLR Binding: Competitive binding assay using native LDL



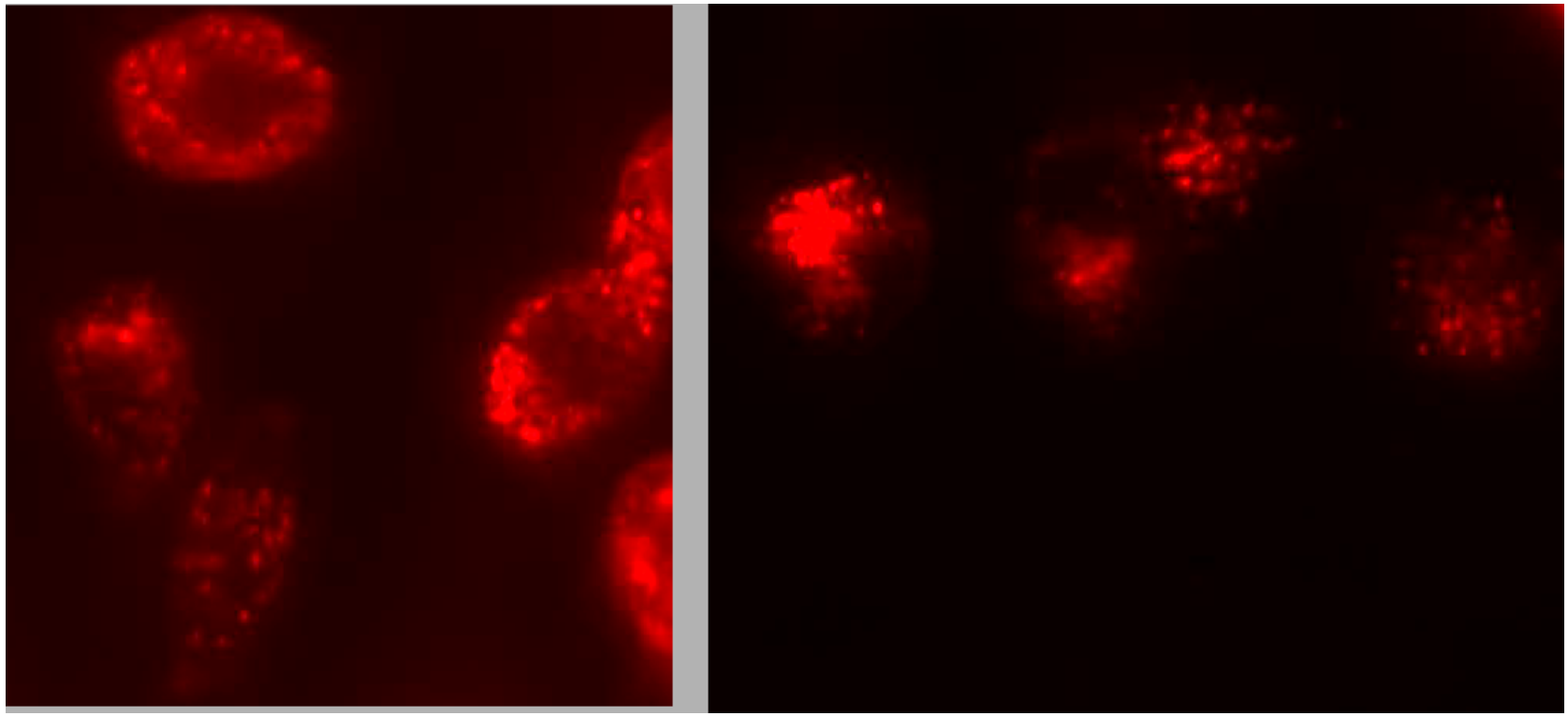
Native LDL-DiI

Native LDL-DiI +
100 fold excess sLDL

SF-767: 1hr, 4°C

Specific sLDL Uptake: SF-767 vs. U-251

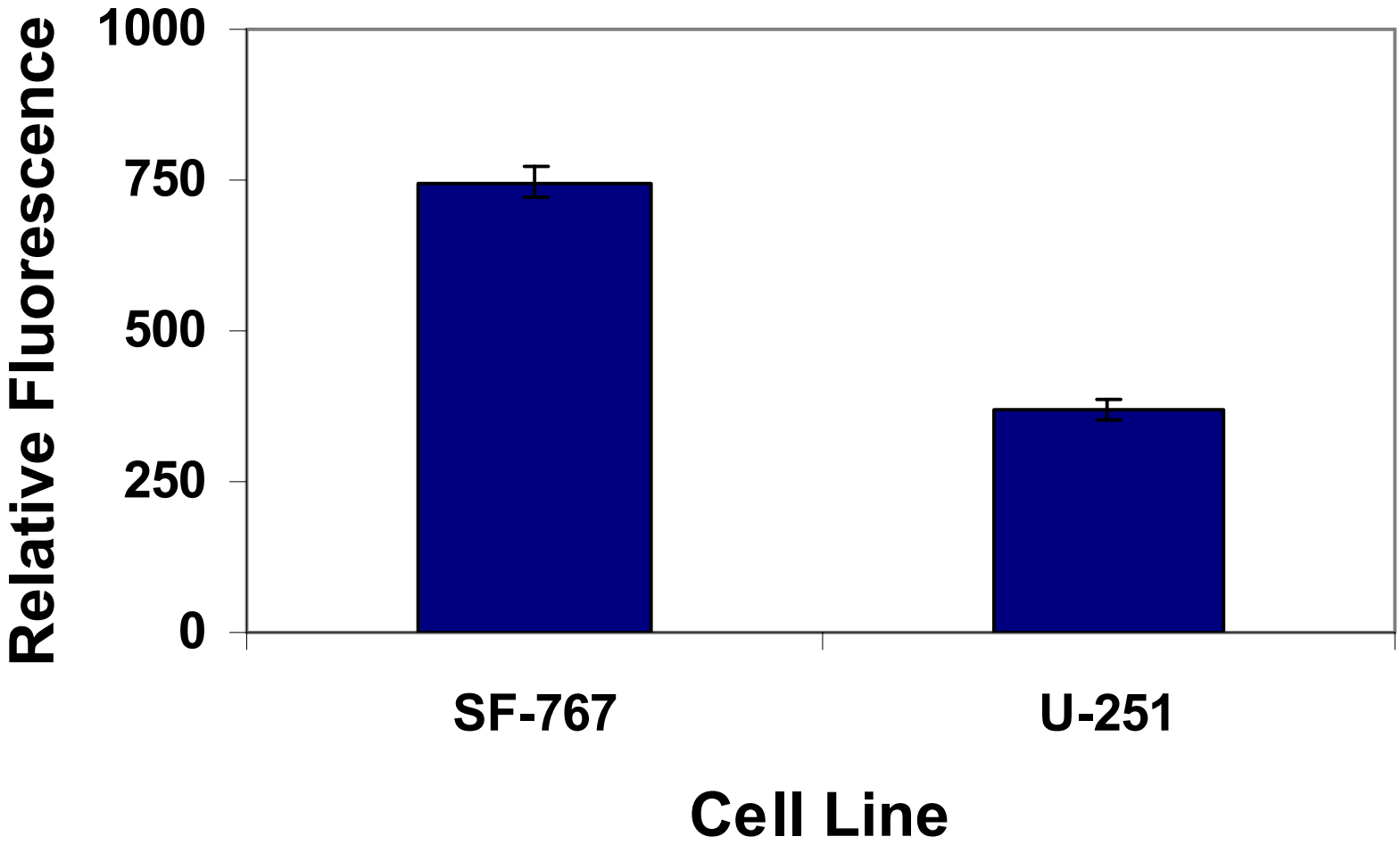
Dependence on receptor number



SF-767 288,000 LDLR U-251 128,000 LDLR

1.5 µM peptide, 3 hrs, 37°C

LDLR-Specific Uptake of nanoLDL: SF-767 vs. U-251

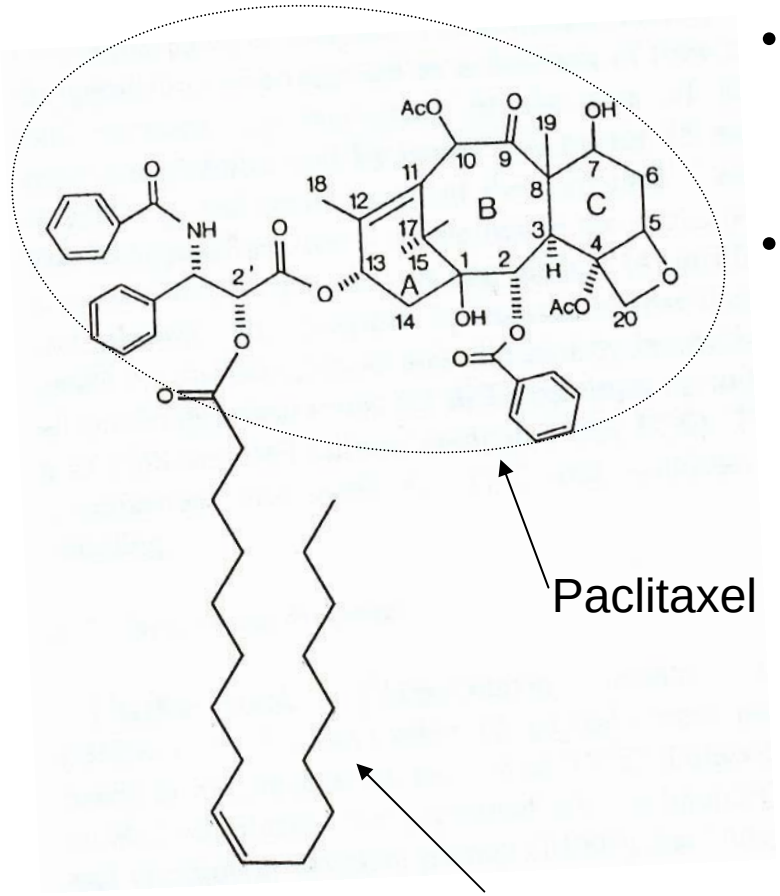


1.5 µM peptide, 3 hrs, 37°C

Conclusions

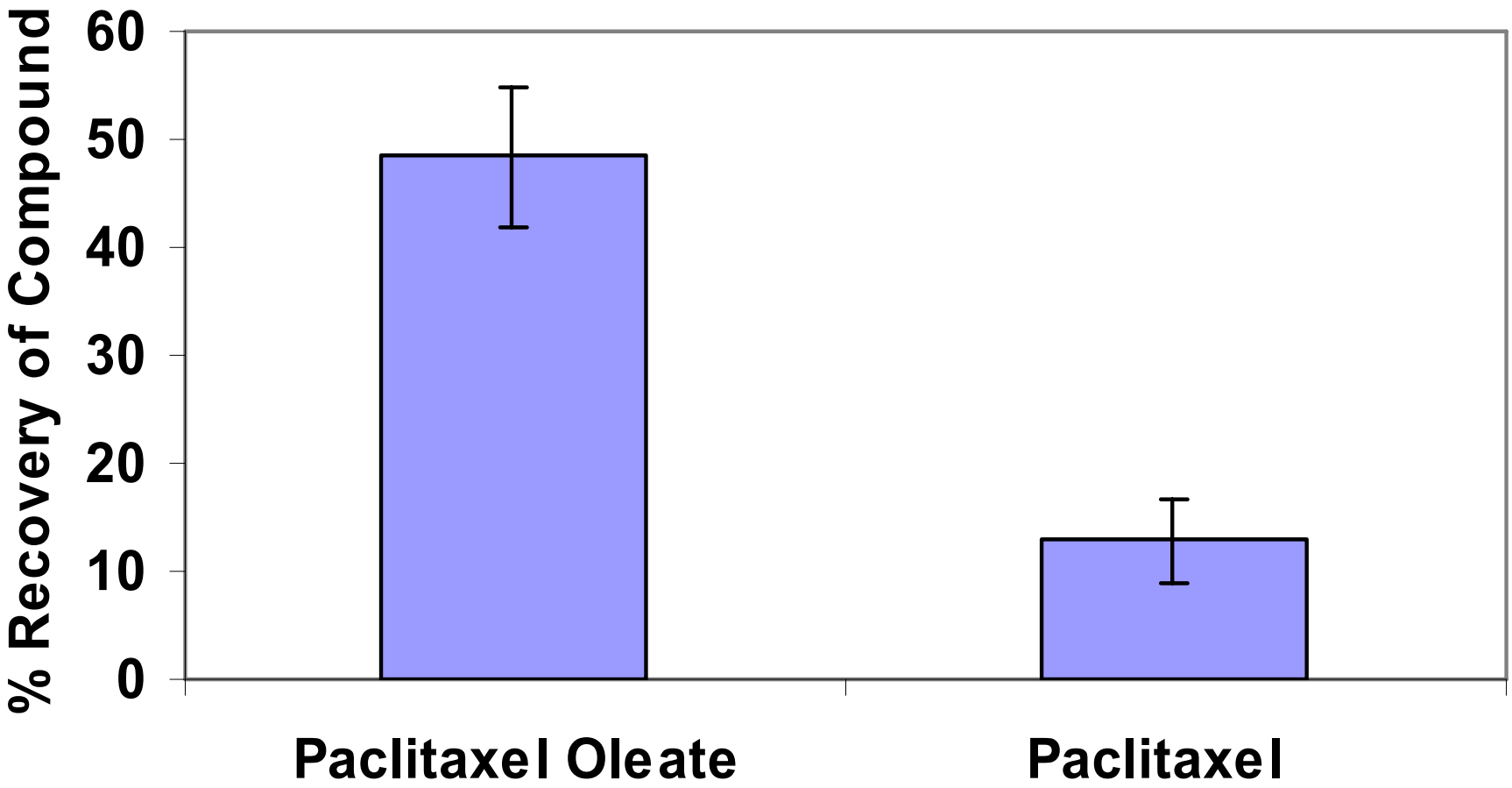
- Nano-LDL constructed with a synthetic peptide containing the LDL receptor binding motif can bind specifically to LDL receptors on GBM.
- Nano-LDLs are internalized into the cell and traffic to the lysosome.
- Question: Can the nano-LDL deliver a lethal dose of anti-cancer drugs to GBM cells?

Paclitaxel Oleate

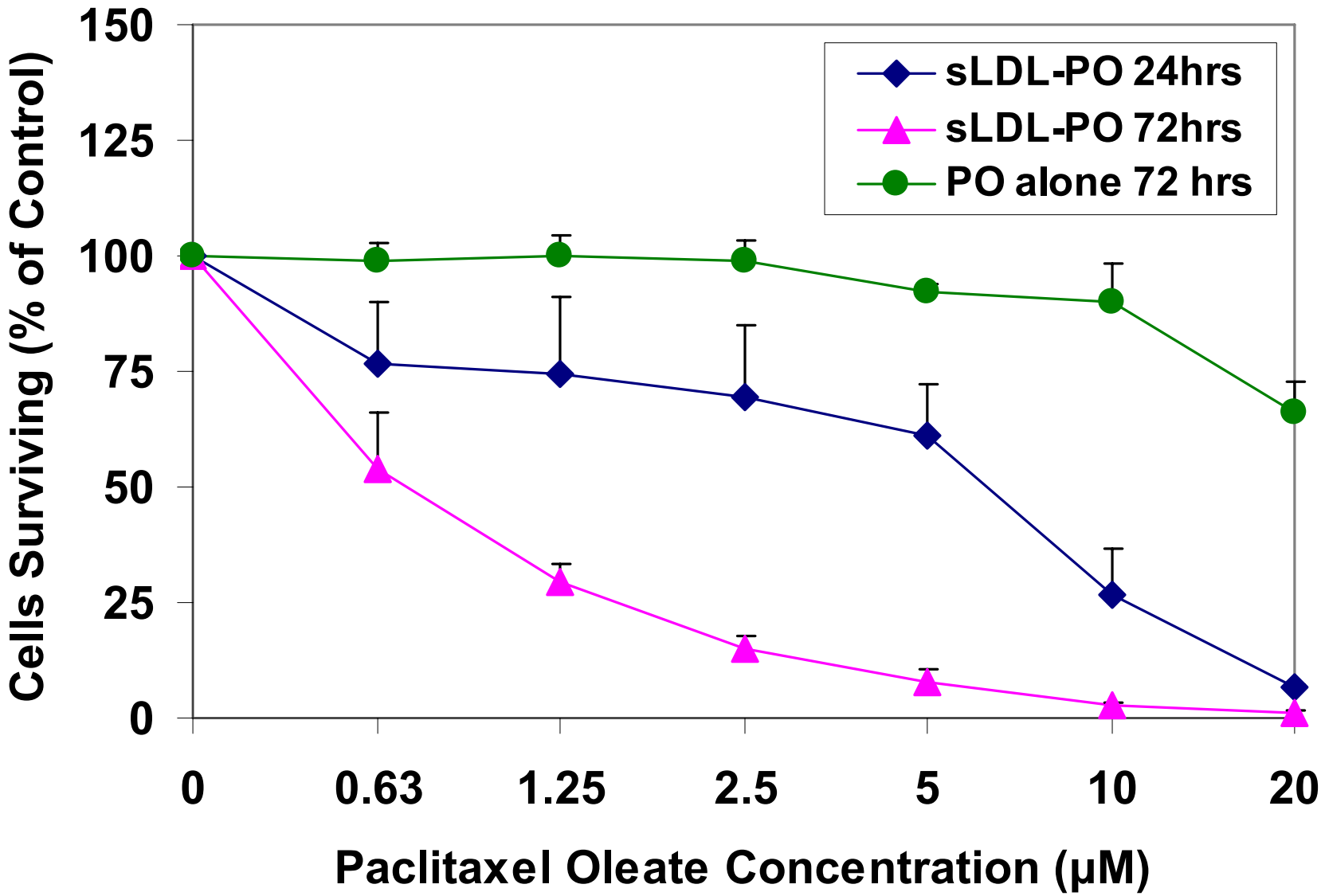


- A chemotherapeutic agent that prevents depolymerization of microtubules during cell division
- Lipophilic oleate chain helps binding to microemulsion

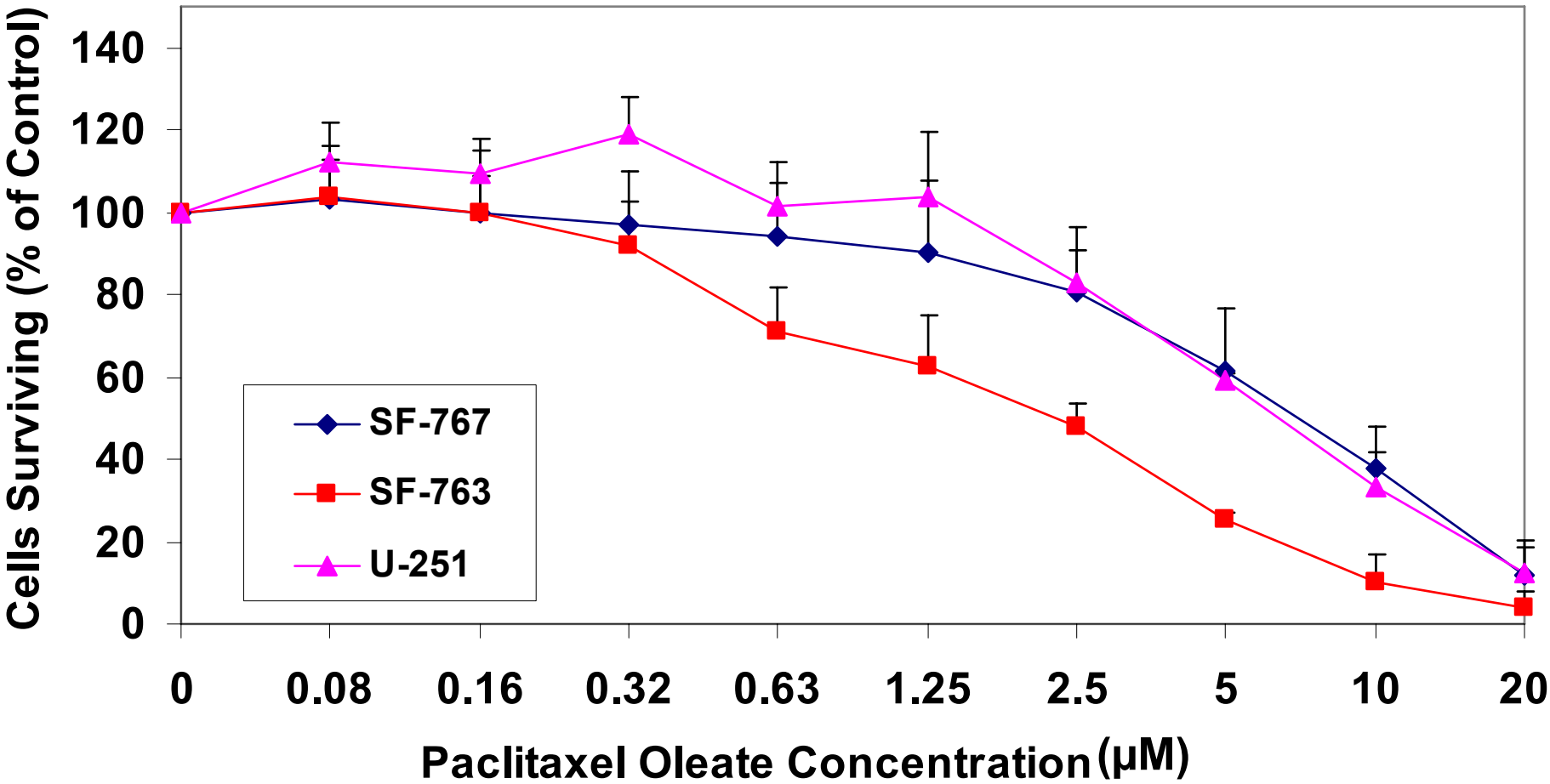
Paclitaxel Oleate vs Paclitaxel Incorporation into Lipid Emulsion



Cell Killing: HeLa Cells

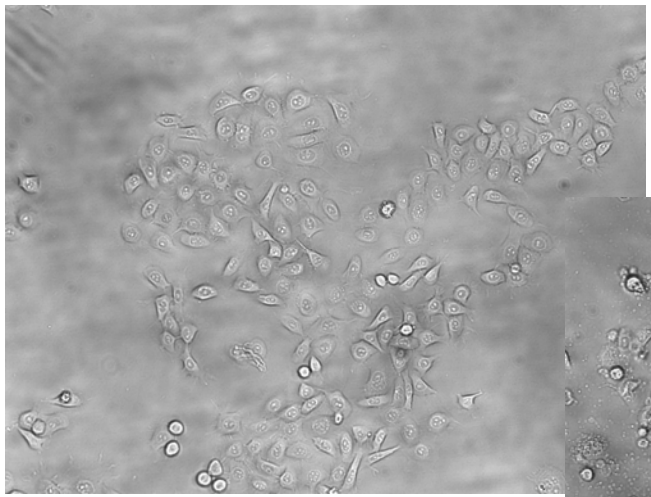


Three Different GBM Cell Lines: Cell Killing Using Paclitaxel Oleate

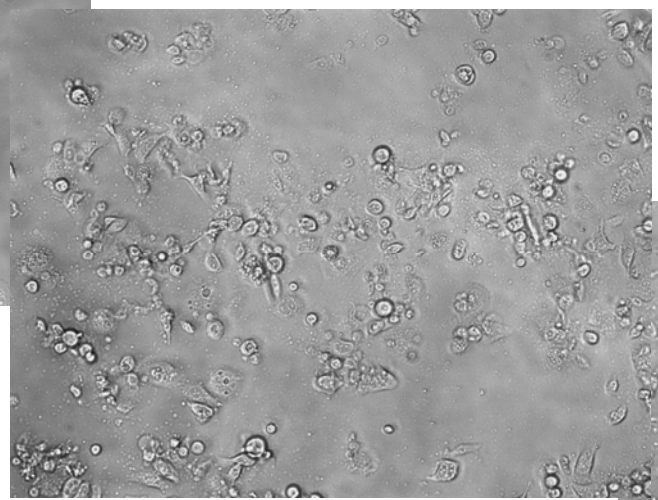


72 hr total incubation: 6hrs with sLDL-PO

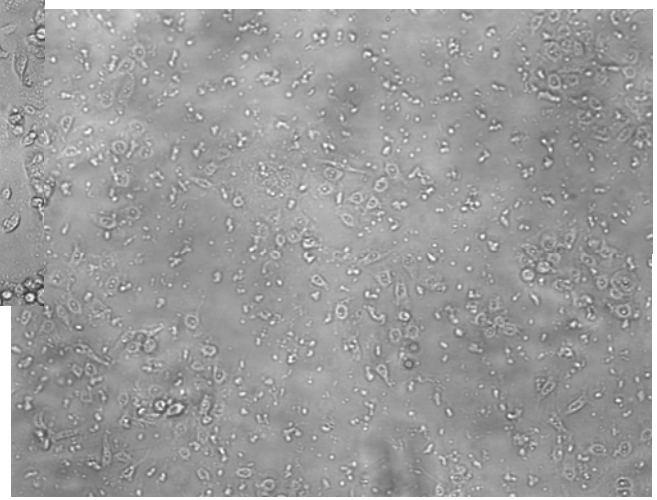
Cell Killing: SF-767 Cells



Start of Experiment



sLDL-PO (5 μM)



sLDL-PO (20 μM)

72 hrs with sLDL-PO

Conclusions

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Paclitaxel oleate

- Succeeded in creating a targeted drug delivery nano-particle directed to GBM via the LDL receptor.
- Nano-LDL particle has the capacity for delivering highly lipophilic drugs.
- Targeted delivery will reduce non-specific toxicity.

Future Directions

- Assess whether LDLR is upregulated in other types of CNS and spinal cord tumors in adults and children.
- Target alternative receptors.
- Improve cell killing; use more toxic drugs.
- In vivo studies.

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