

# Novel anti-tumour agents targeting the cell membrane

Marcel Verheij

NETHERLANDS  
CANCER  
INSTITUTE



ANTONI VAN LEEUWENHOEK



*Uniting physics, biology and medicine for better healthcare*



# Disclosures

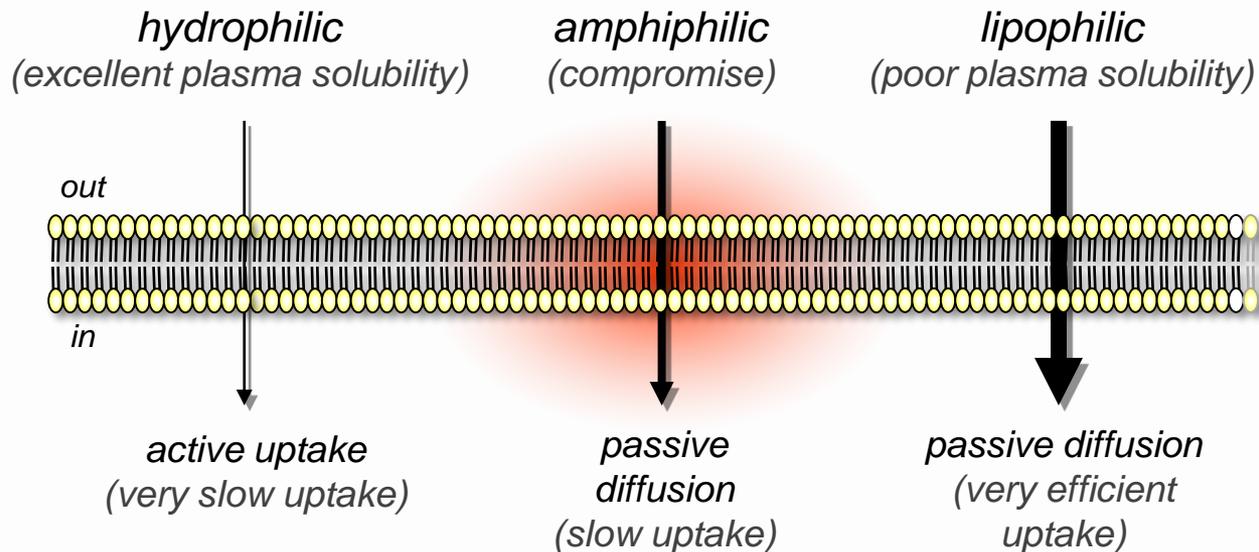
Research funding:

- *Elekta*
- *Roche*
- *Astra Zeneca*
- *CellProtect*
- *AbbVie*

# Plasma membrane

## Barrier and target for cancer therapy

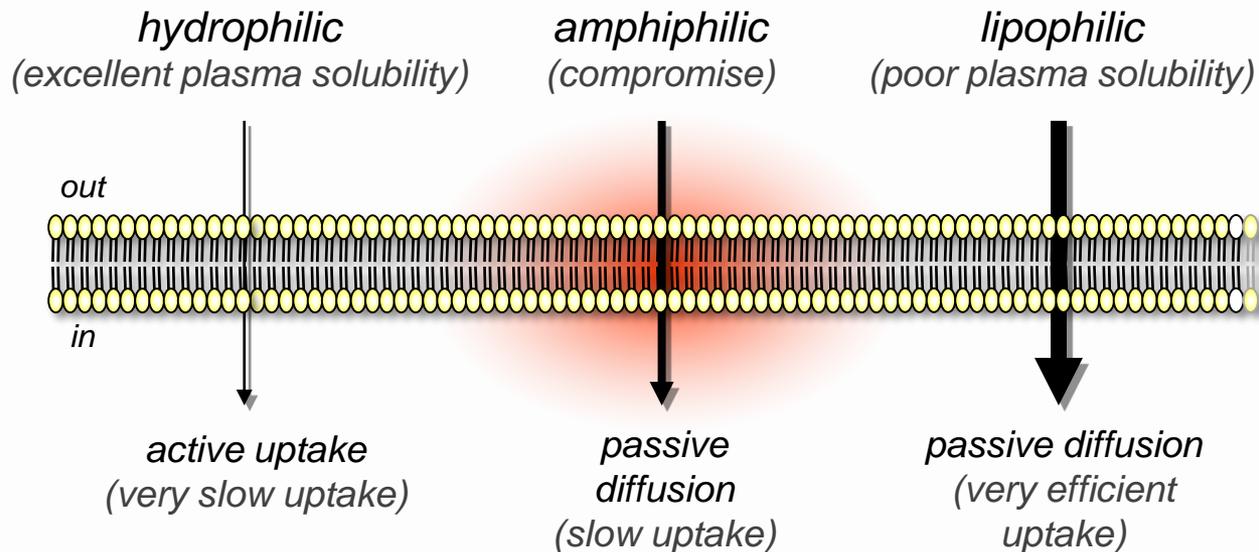
- Single membrane to cross
- Sphingolipids & Sterols: BARRIER



# Plasma membrane

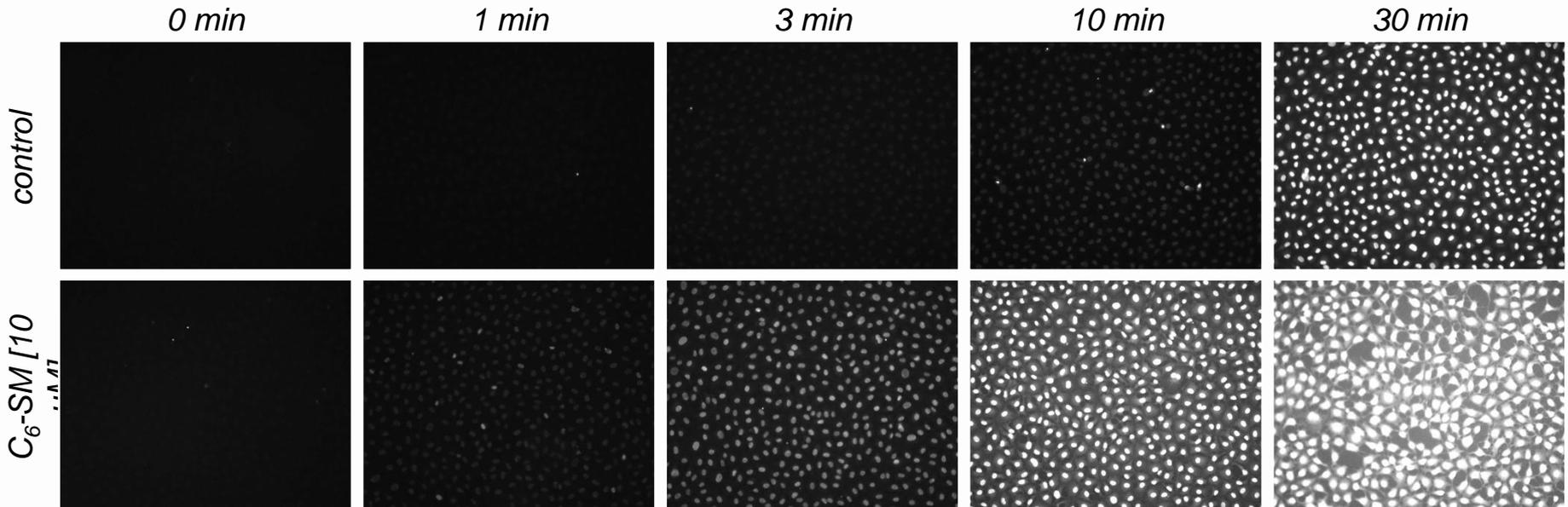
## Barrier and target for cancer therapy

- Single membrane to cross
- Sphingolipids & Sterols: BARRIER



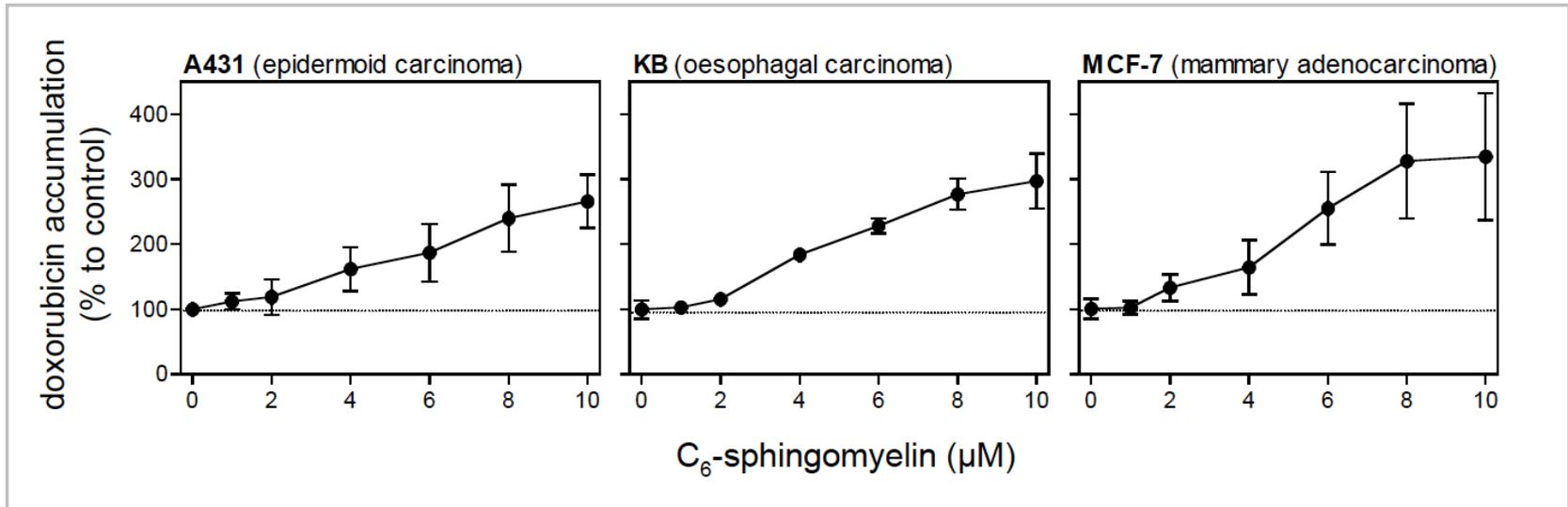
*Can we modulate membrane barrier function to improve intracellular drug delivery?*

# Serendipity: sphingolipids enhance intracellular doxorubicin accumulation and cytotoxicity



*Bovine  
Aortic Endothelial Cells (BAEC)*

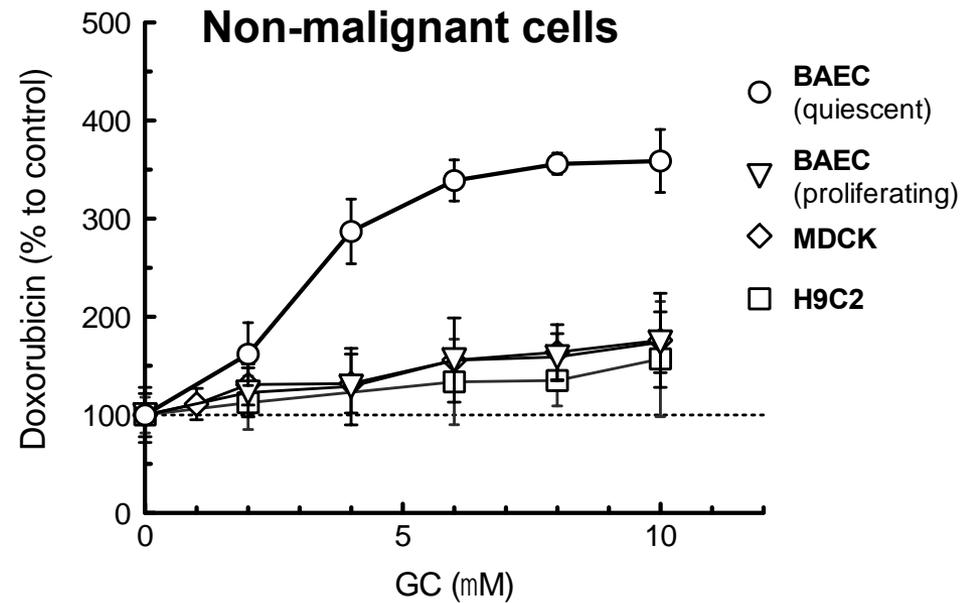
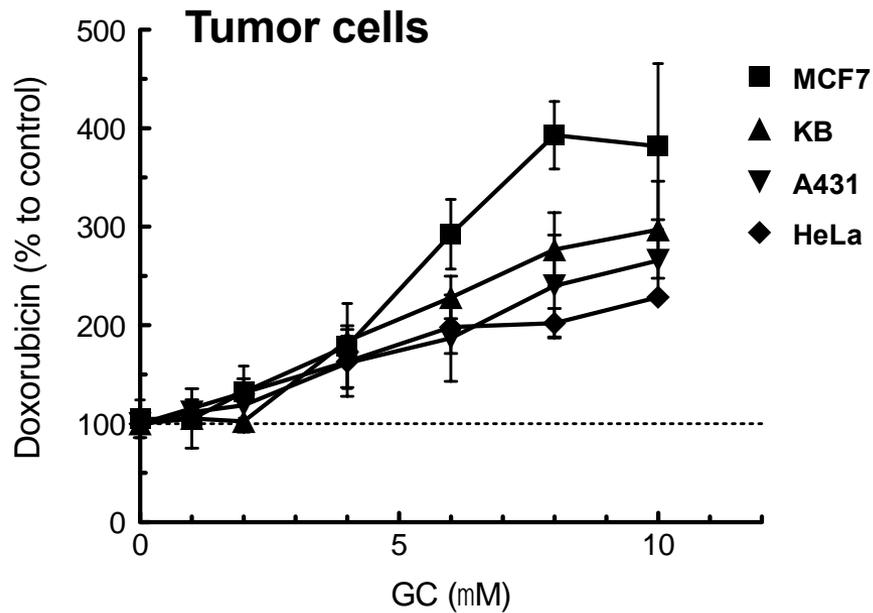
# SCS act at the plasma membrane level in multiple tumor cell types



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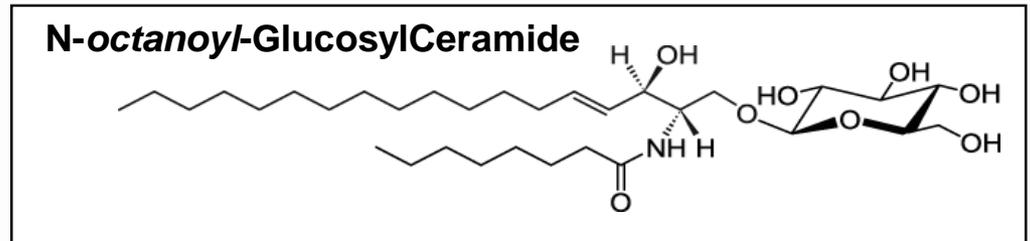


# SCS act preferentially on tumor cells



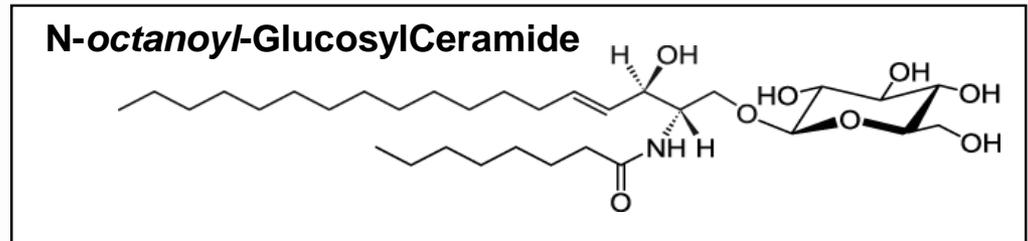
# Short-Chain Sphingolipids (SCS)

- Analogues of natural lipids
- Truncated acyl chains



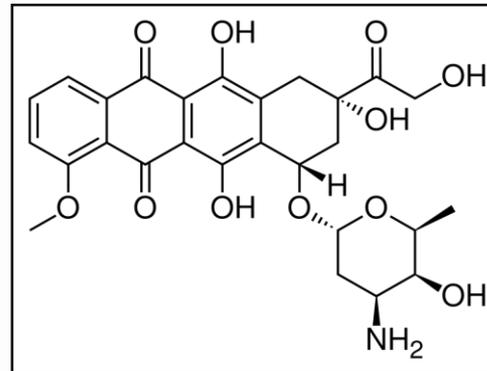
# Short-Chain Sphingolipids (SCS)

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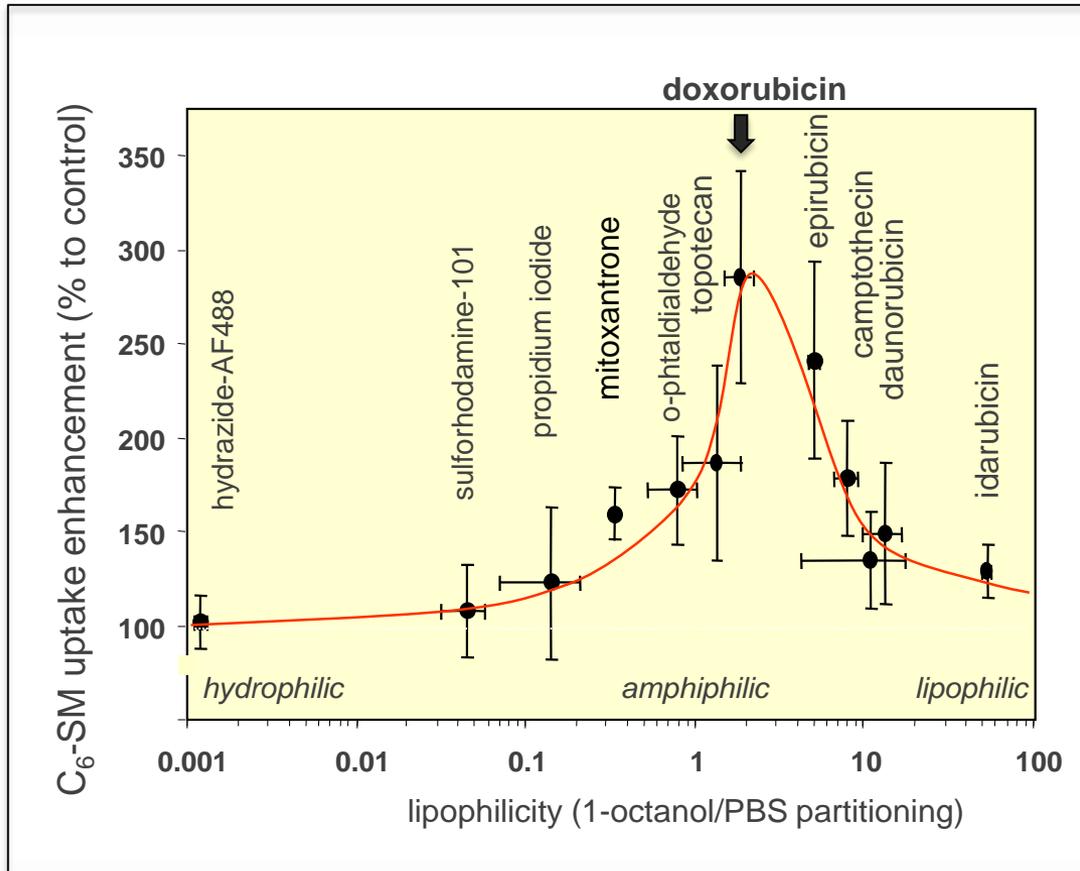
## Doxorubicin

- Widely used anthracycline
- Fluorescent
- Adriamycin™
- Caelyx® / Doxil®- Liposomal formulations



# Optimal combination of drug amphiphilicity and lipid requirements

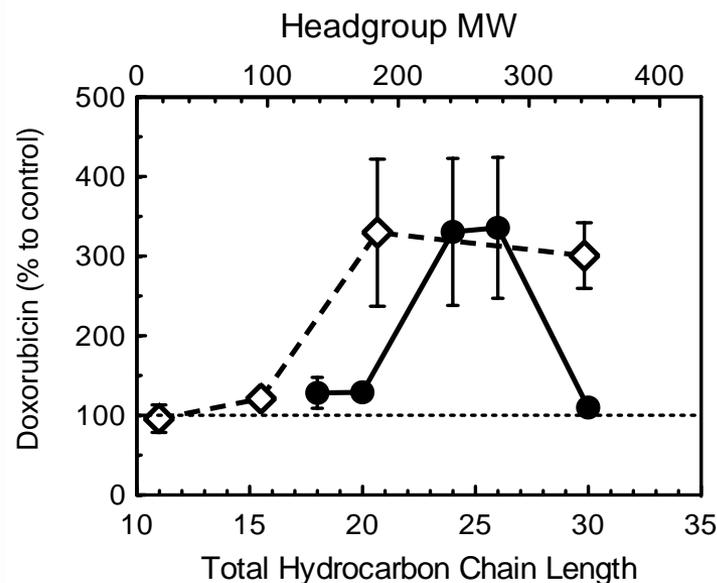
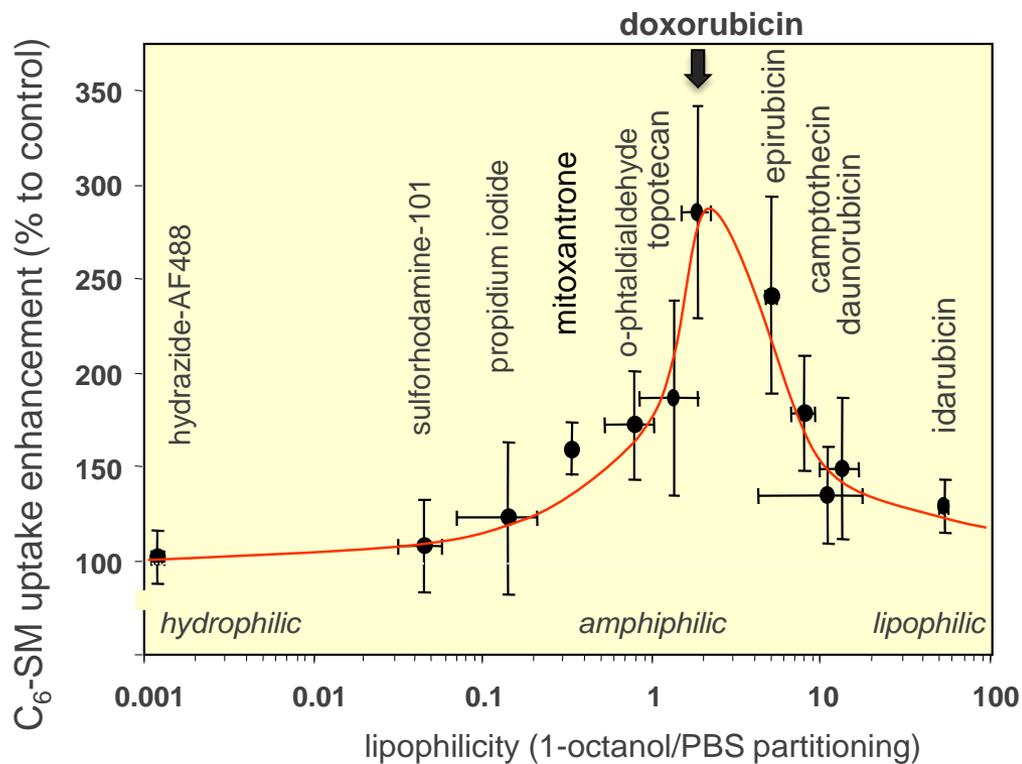
Compound (drug)



# Optimal combination of drug amphiphilicity and lipid requirements

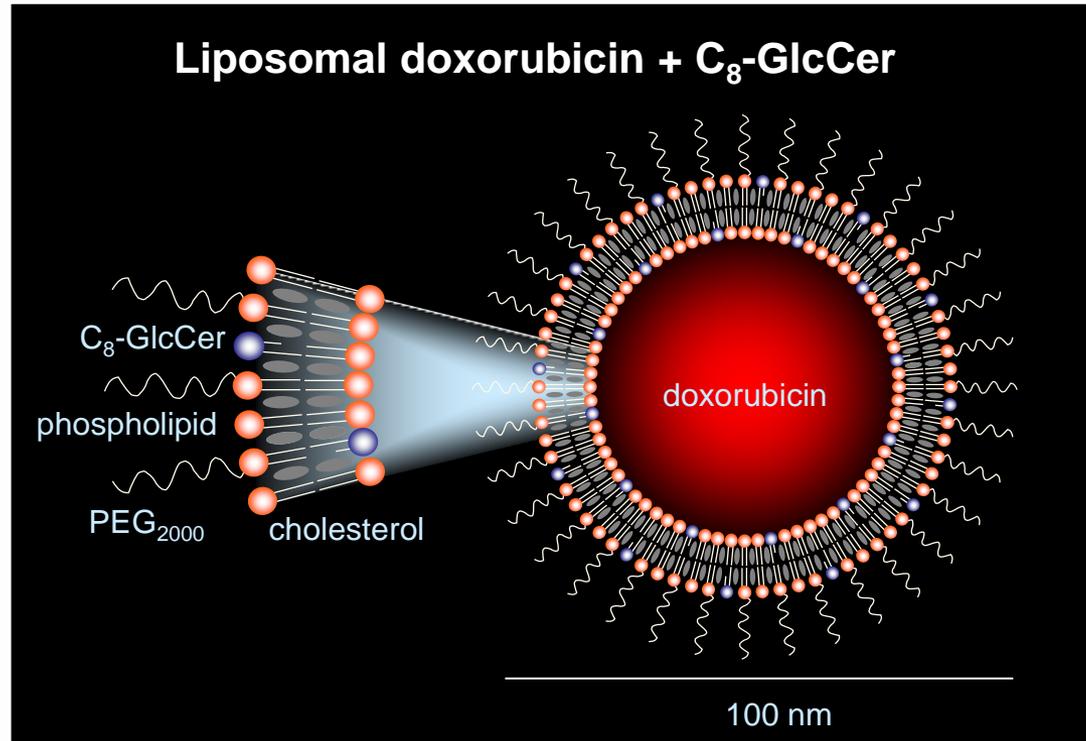
Compound (drug)

Sphingolipid



- Optimal hydrocarbon chain length (20-30)
- Minimum head group size (>100 Da)
- Independent on stereochemistry

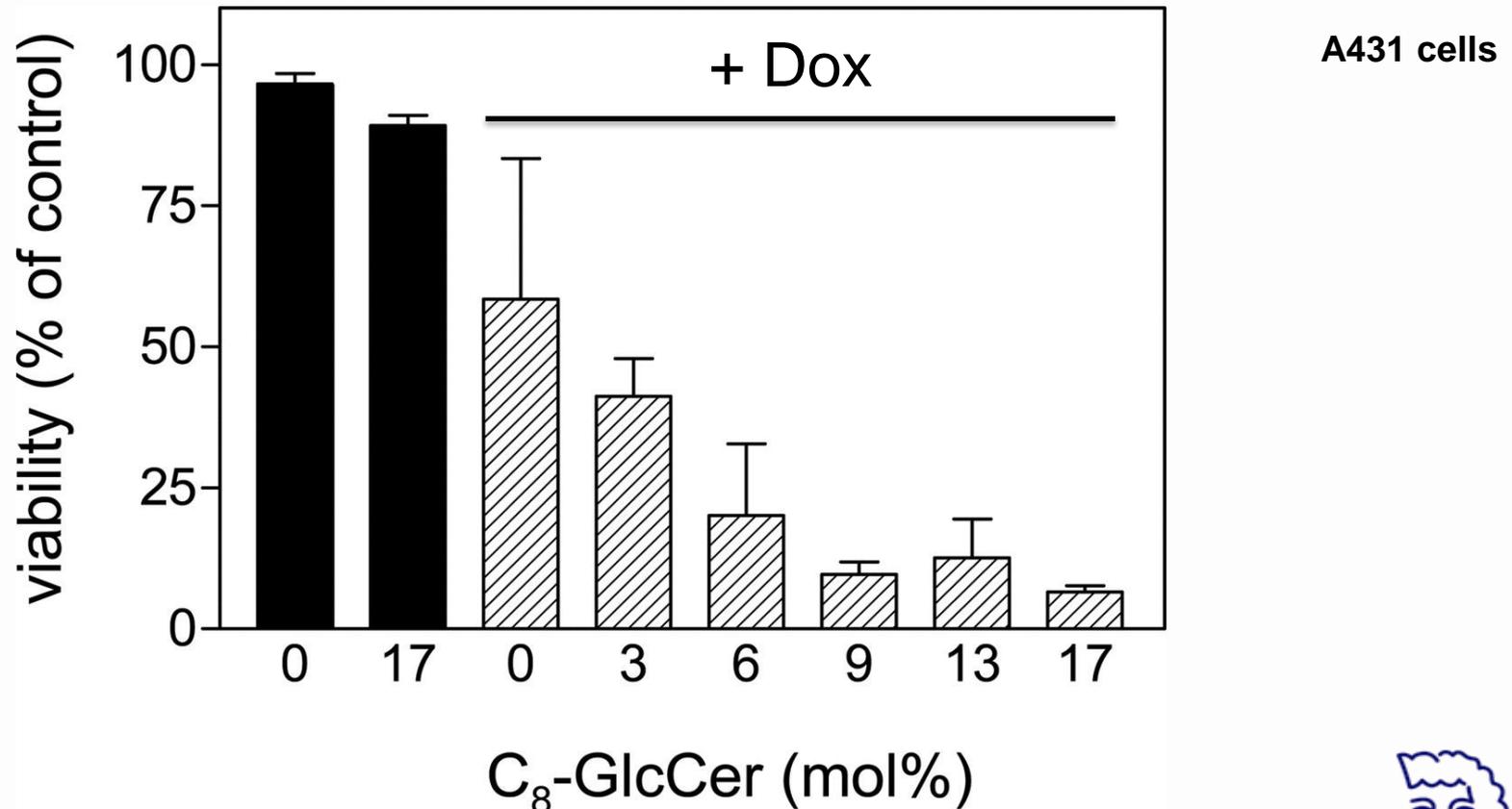
# GC-Lipodox<sup>®</sup>: co-formulation of GC and pegylated liposomal doxorubicin



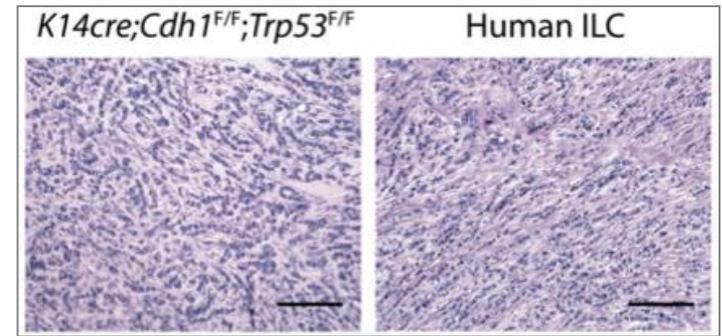
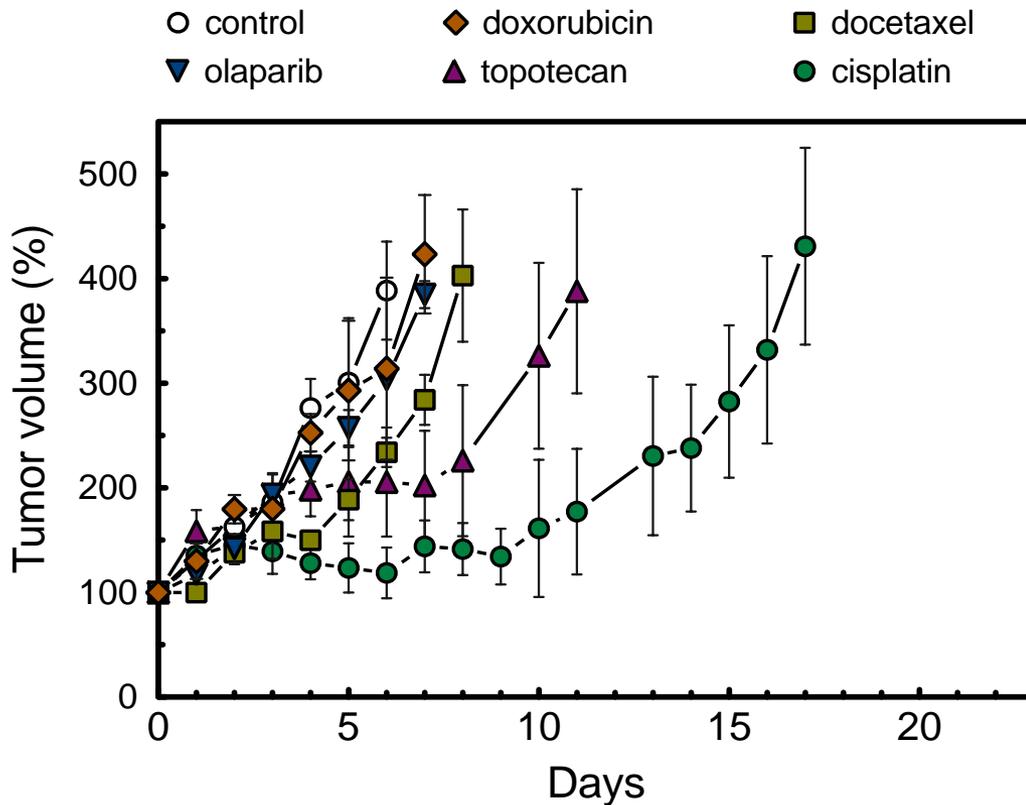
Particle characteristics unaffected by GC:

- Size, polydispersity, loading, stability (up to 6 months)

# GC co-formulation improves cytotoxic efficacy of liposomal doxorubicin



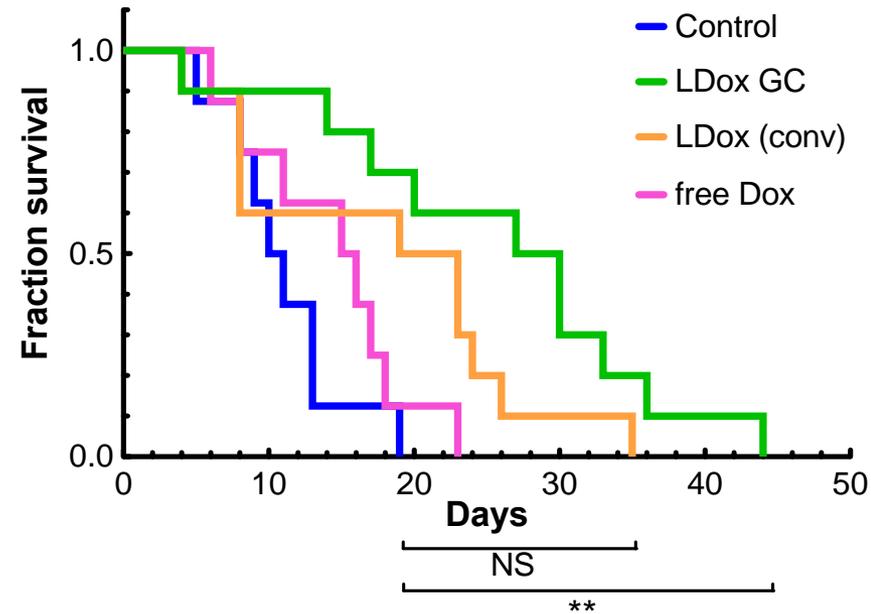
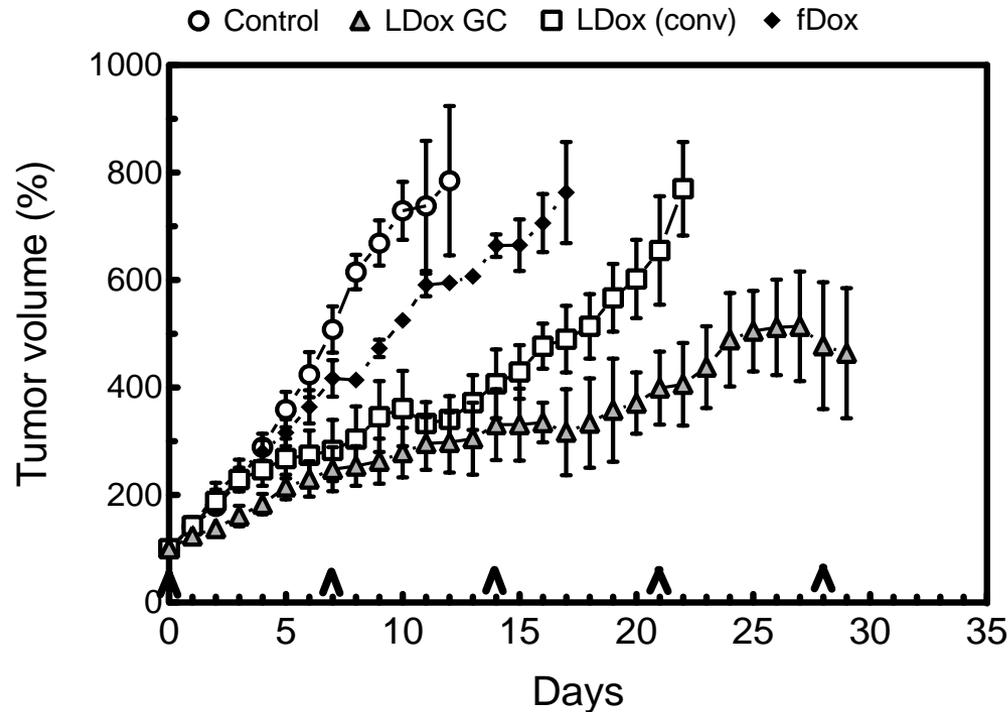
# Model: multidrug resistant $Cdh1^{-/-};p53^{-/-}$ mouse mammary tumors



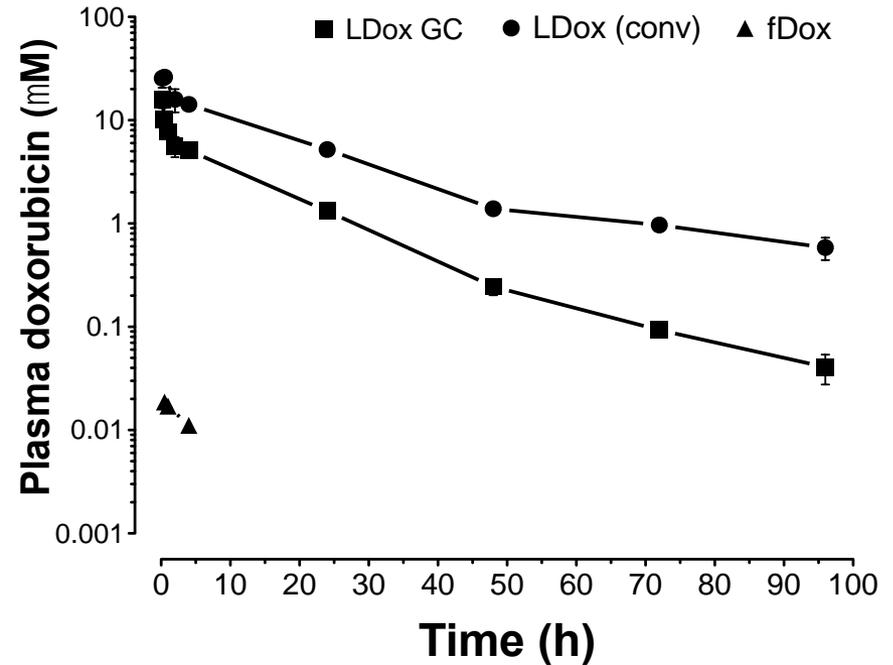
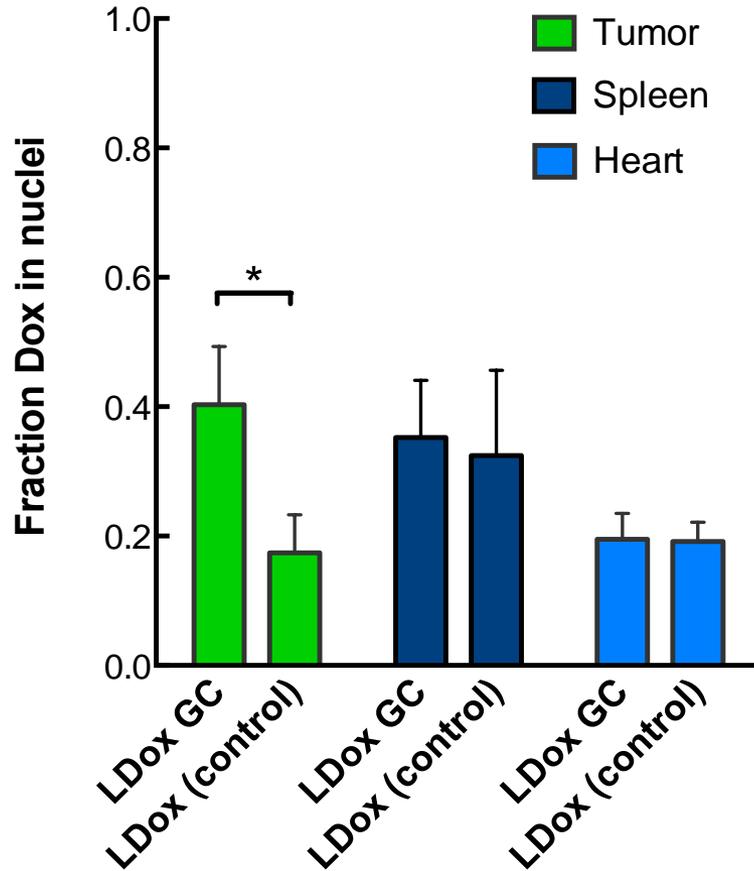
*Derksen PW, Jonkers J, et al. Cancer Cell (2006)*

All treatments at Maximum Tolerated Dose

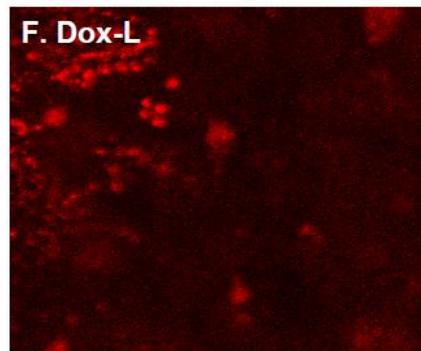
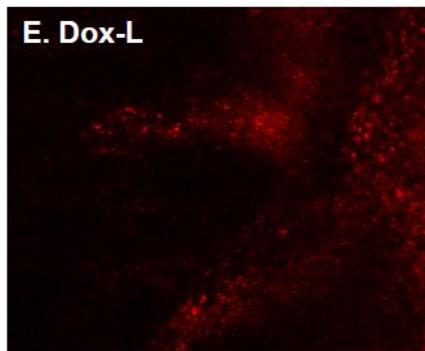
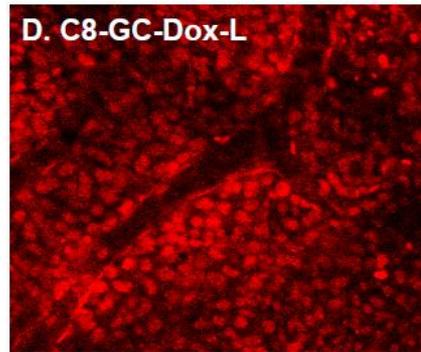
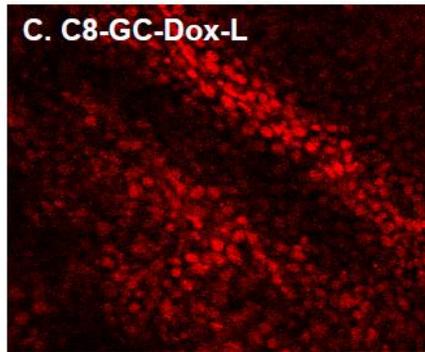
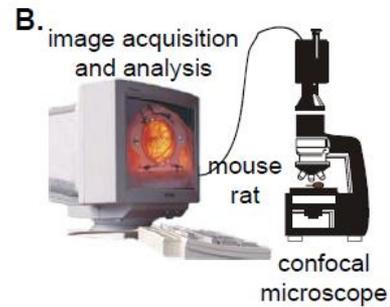
# GC-Lipodox: Co-administration of GC establishes superior anti-tumor efficacy



# GC-Lipodox: enhanced tumor accumulation and reduced systemic exposure



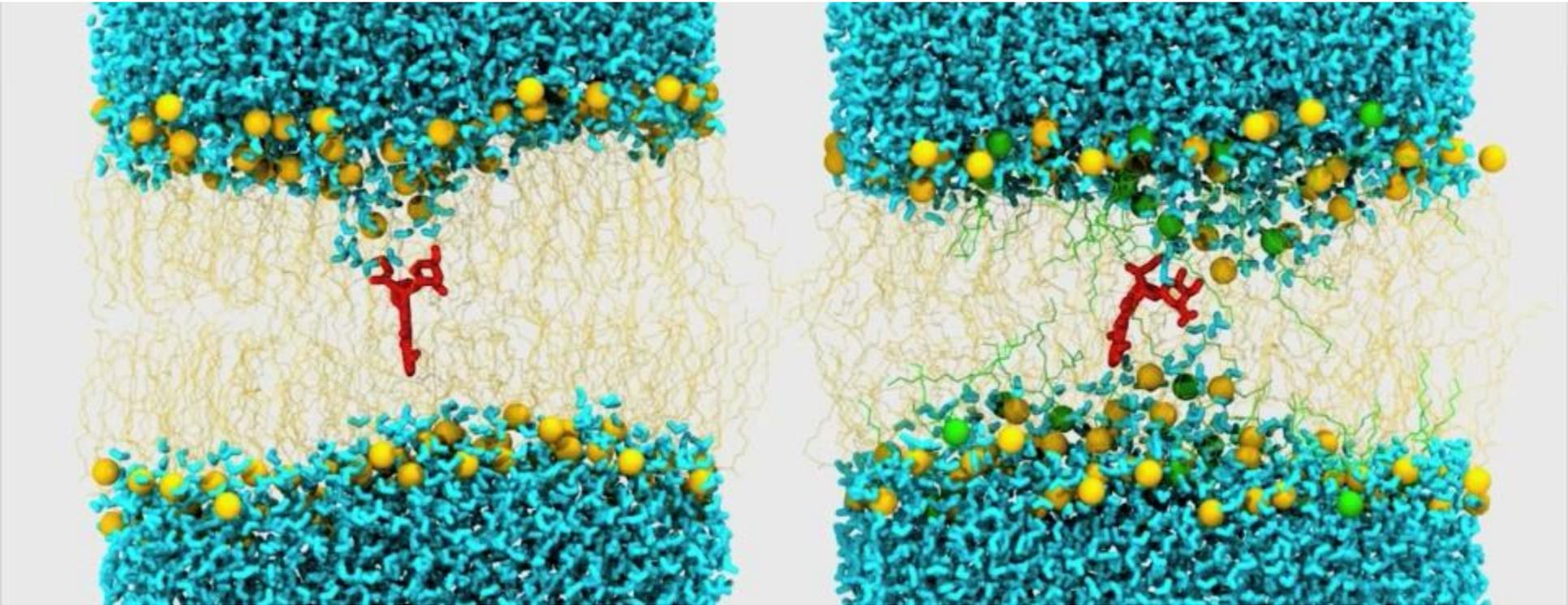
# GC-Lipodox: enhanced tumor accumulation



*B16 melanoma model (intravital microscopy)*

# SCS assemble transient membrane gateways

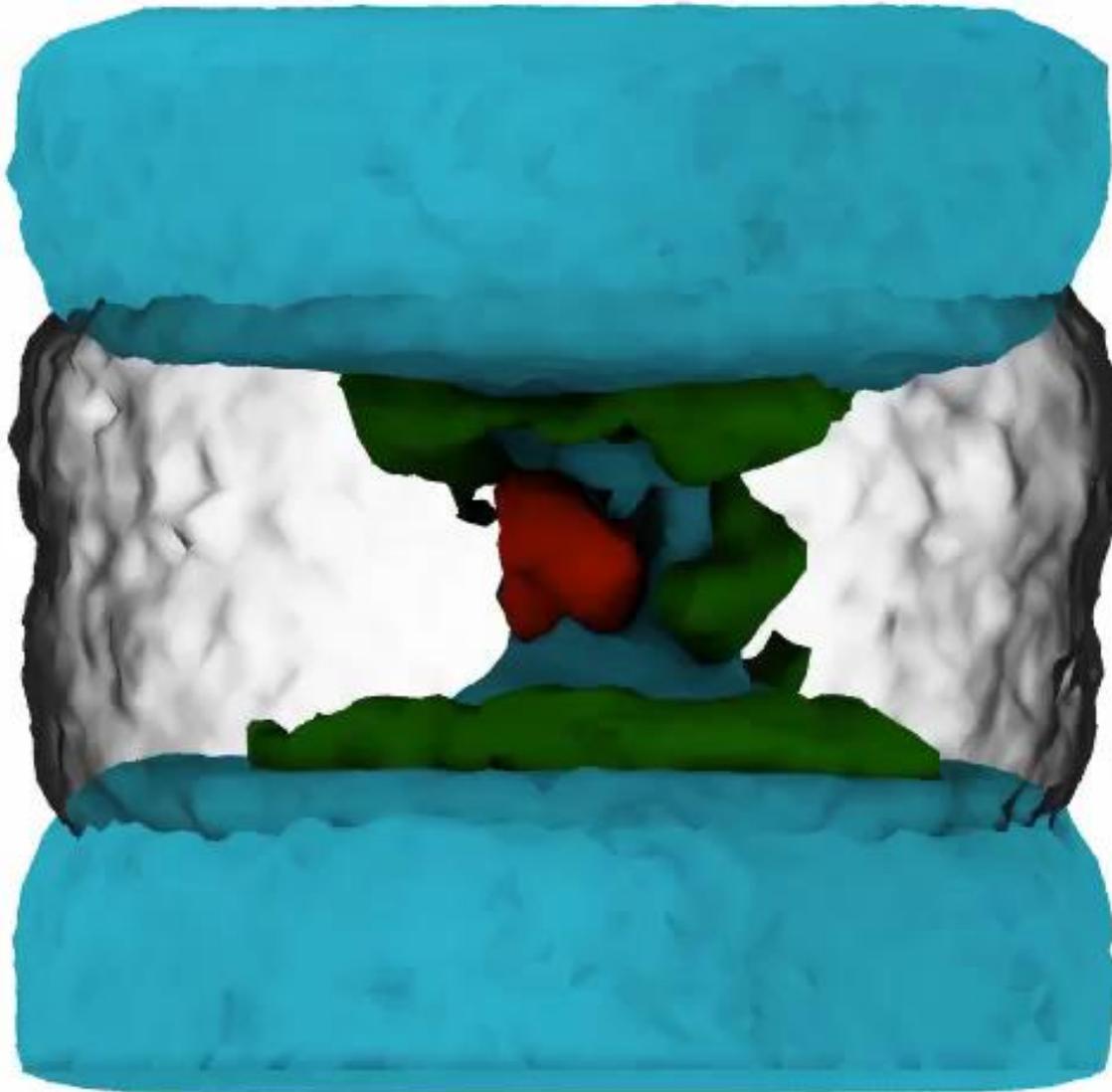
*Molecular dynamics simulation (in silico): Doxorubicin*



## *Control*

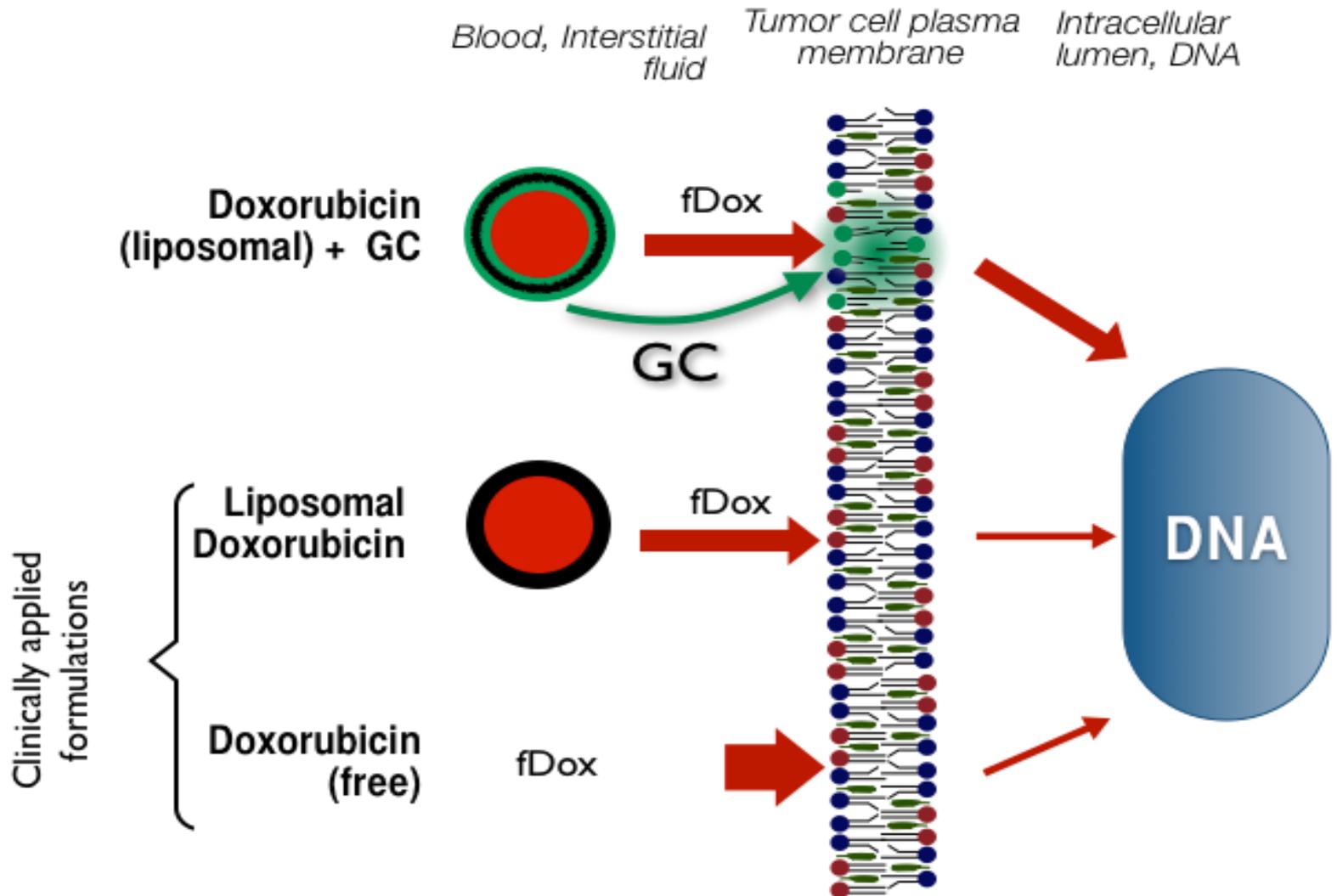
- Bilayer: 144 lipid molecules (control)
- Bilayer: 114 lipid and 30 short-chain lipid molecules (20 mol%)
- GROMOS united-atom force field
- 250 nanoseconds simulation
- Doxorubicin in the lipid bilayer > membrane translocation

## +SCS



-  Natural Lipid (translucent)
-  Short Chain Lipid
-  Doxorubicin
-  Water

# GC-Lipodox: increased therapeutic window



# Screen for other compounds

## Drug Compound Libraries

- NCI-88 oncology set: 88 clinically approved anti-cancer drugs (*NCI, Bethesda, USA*)
- Selleck-140 kinase inhibitors library (*SelleckChem, TX, USA*)

## Robotic Platform

- STARlet Replicator Platform (*Hamilton Robotics, NV, USA*)



**Cell lines:** WEP3, A431, HCT116

## Short Chain Sphingolipids

- C8-GlcCer (sphingolipid)
- C9-PC, C10-PC, C11-PC (glycerophospholipids)

# Hits from kinase inhibitor screen

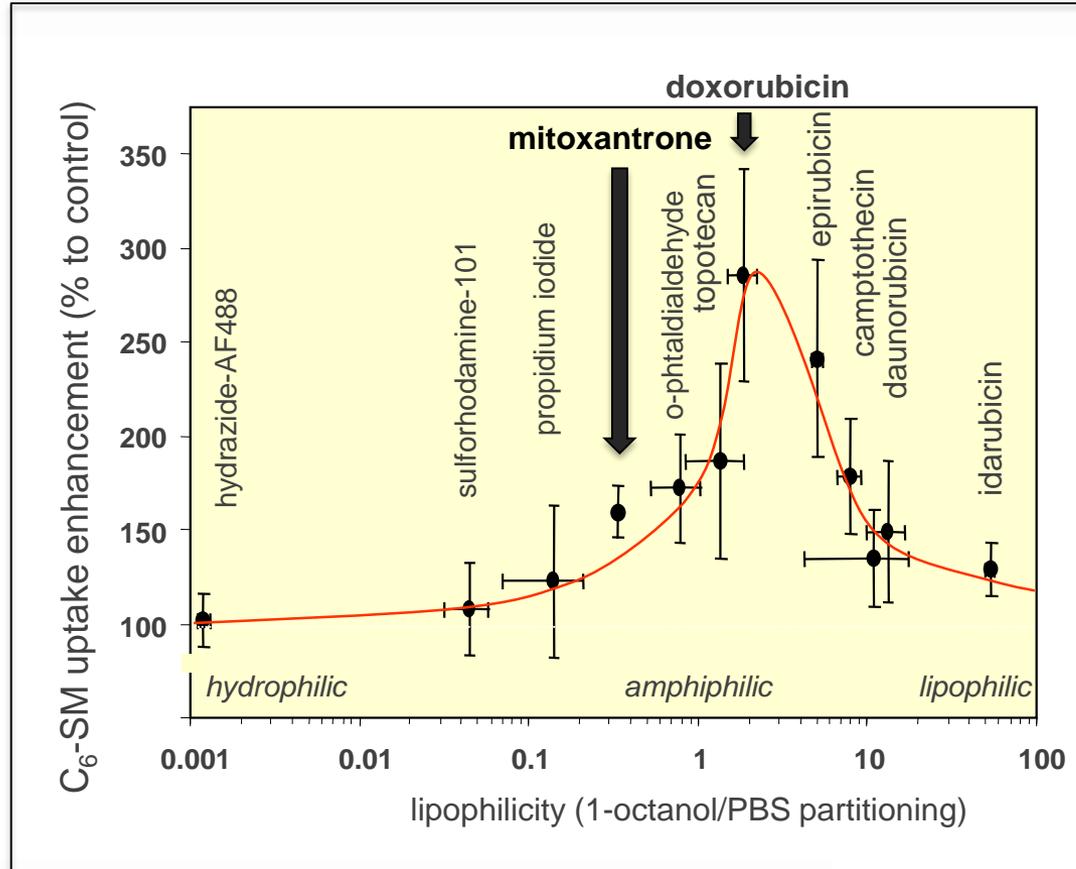
Drug	Max IC shift (log)	Average IC shift (log)	Molecular Target
PIK-75	2.3	1.7	PI3K, DNA-PK
GSK1059615	1.7	1.4	PI3K, mTOR
ZSTK474	1.5	1.1	PI3K
GDC-0941	1.2	1.0	PI3K
AZD6482	1.1	0.8	PI3K
Danuserib (PHA-739358)	1.1	0.8	Aurora Kinase, FGFR, Bcr-Abl, c-RET, Src
PHA-793887	1.0	0.7	CDK
PIK-93	1.0	0.6	PI3K, VEGFR
MK-2206	0.9	0.8	Akt
PIK-90	0.9	0.7	PI3K

*HCT116 cells (human colon carcinoma)*

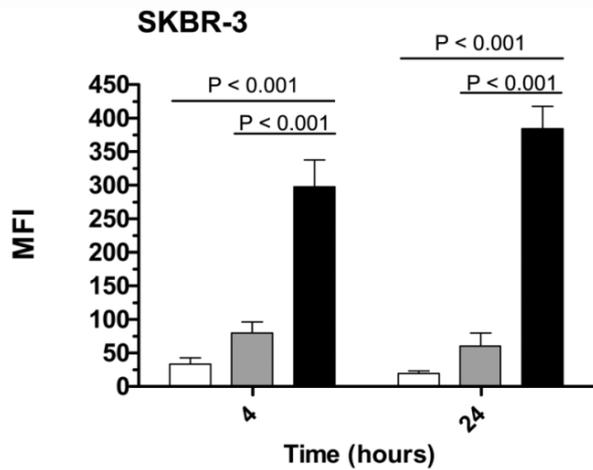
# Hits from NCI-88 drug screen

Drug	Molecular Weight (Da)	Max IC shift (log)	Average IC shift (log)	Cells
Dactinomycin	1255	1.9	1.4	A431, <u>WEP3</u>
Doxorubicin HCl	543	1.8	1.3	A431, <u>WEP3</u>
Vincristine sulfate	825	1.5	1.3	WEP3
ABT737	813	1.4	1.0	A431
Melphalan	305	1.3	0.8	WEP3
Lapatinib	581	1.2	0.8	A431
<b>Mitoxantrone</b>	444	0.9	0.8	<u>A431, WEP3</u>
Plicamycin	1085	0.9	0.8	<u>A431, WEP3</u>
Nilotinib	530	0.9	0.7	WEP3
Mitomycin C	334	1.2	0.7	WEP3

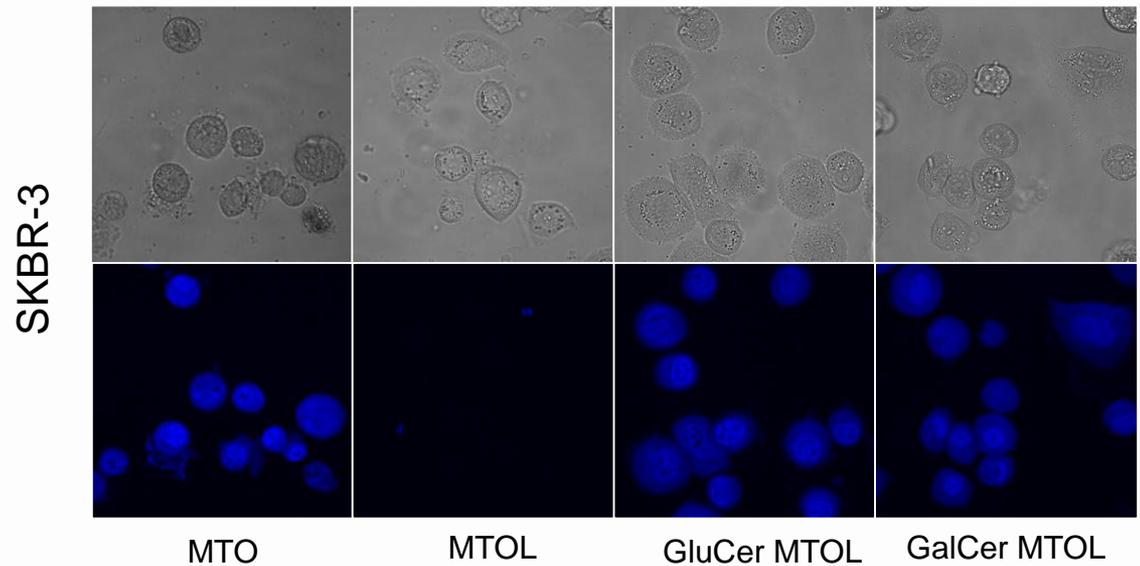
# Optimal drug amphiphilicity: mitoxantrone



# Co-formulation of SCS elevates *in vitro* mitoxantrone accumulation

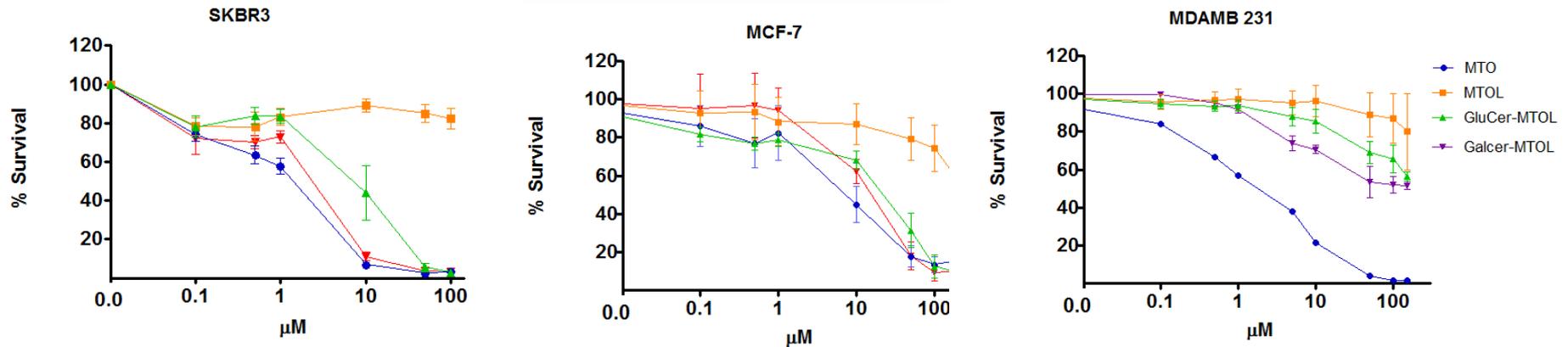


- Standard-MTOL
- GluCer-MTOL
- GalCer-MTOL

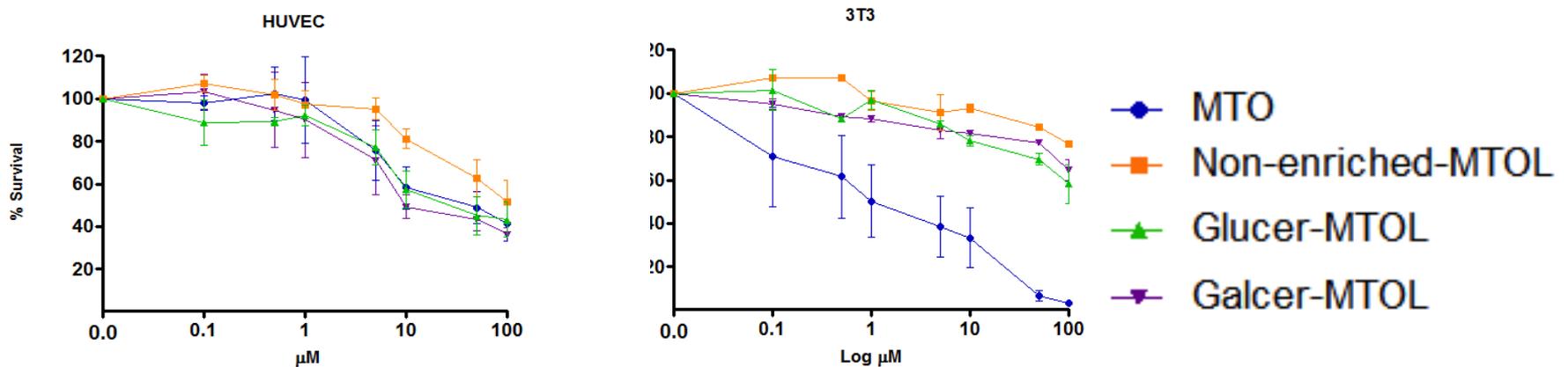


# Elevated MTO accumulation results in improved *in vitro* cytotoxicity

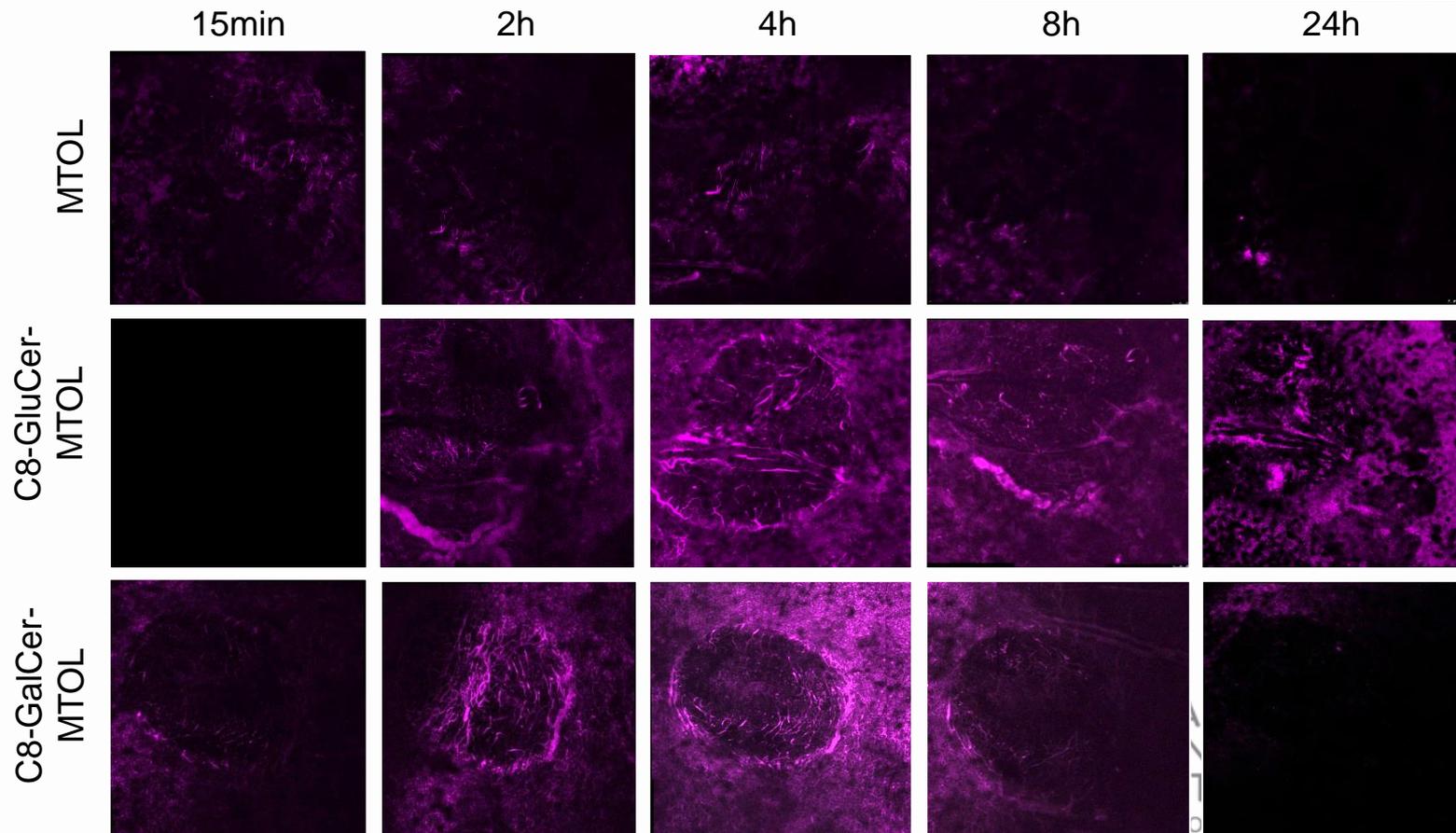
## Tumor Cells



## Normal Cells



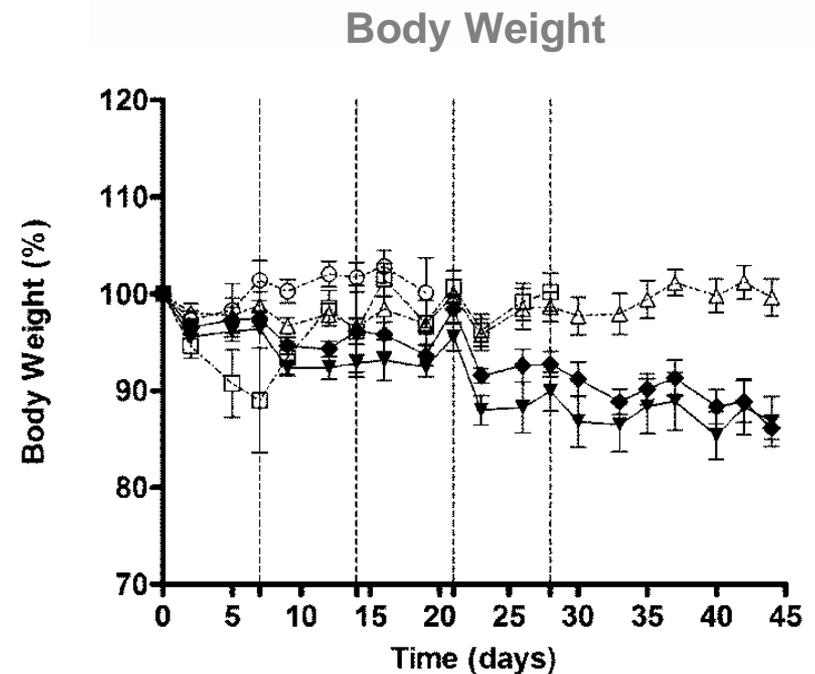
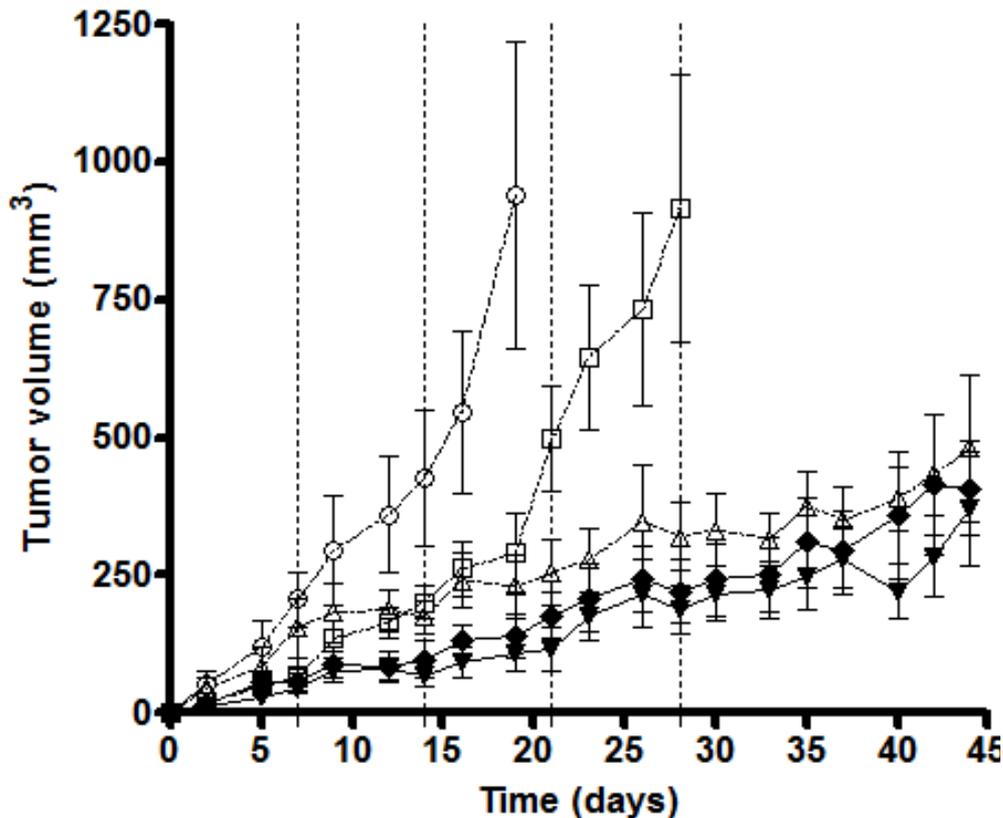
# SCS elevate intratumoral MTO accumulation *in vivo*



MDAMB-231, breast carcinoma

Cordeiro Pedrosa et al. 2015

# Anti-tumor efficacy is improved by SCS MTO liposomes



PBS( $\circ$ ) MTO( $\square$ ) MTOL( $\triangle$ ) C<sub>8</sub>GluCer-MTOL( $\blacktriangle$ ) C<sub>8</sub>GalCer-MTOL ( $\blacktriangledown$ )

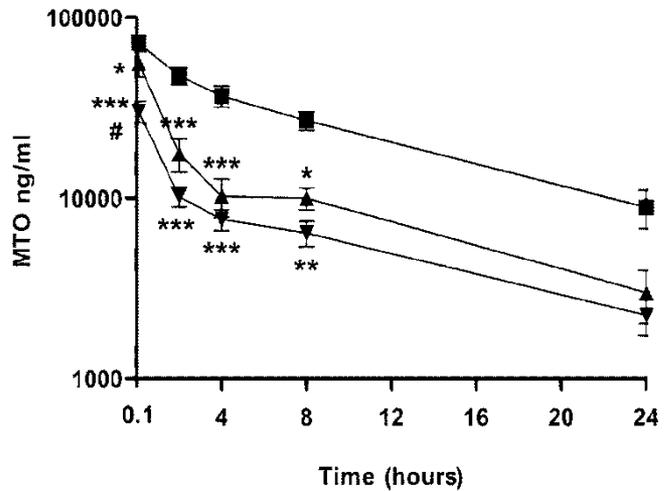
MDAMB-231 orthotopic breast carcinoma

Cordeiro Pedrosa et al. 2015

# SCS reduce MTO plasma levels and skin accumulation



Palmo-plantar erythrodysesthesia



MTOL (■) C<sub>8</sub>GluCer (▲) C<sub>8</sub>GalCer (▼)

PBS



GluCer-MTOL



MTO



MTOL

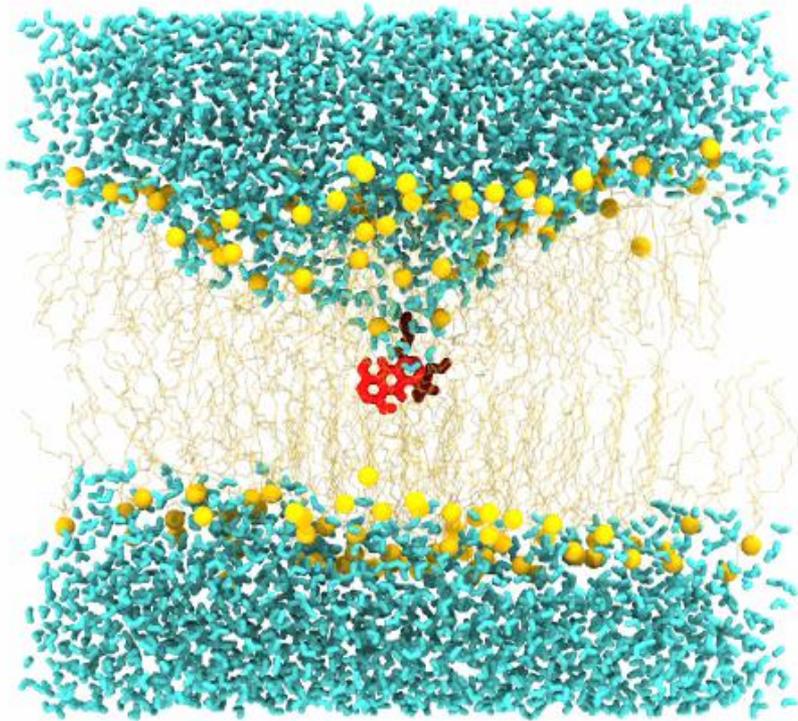


GalCer-MTOL

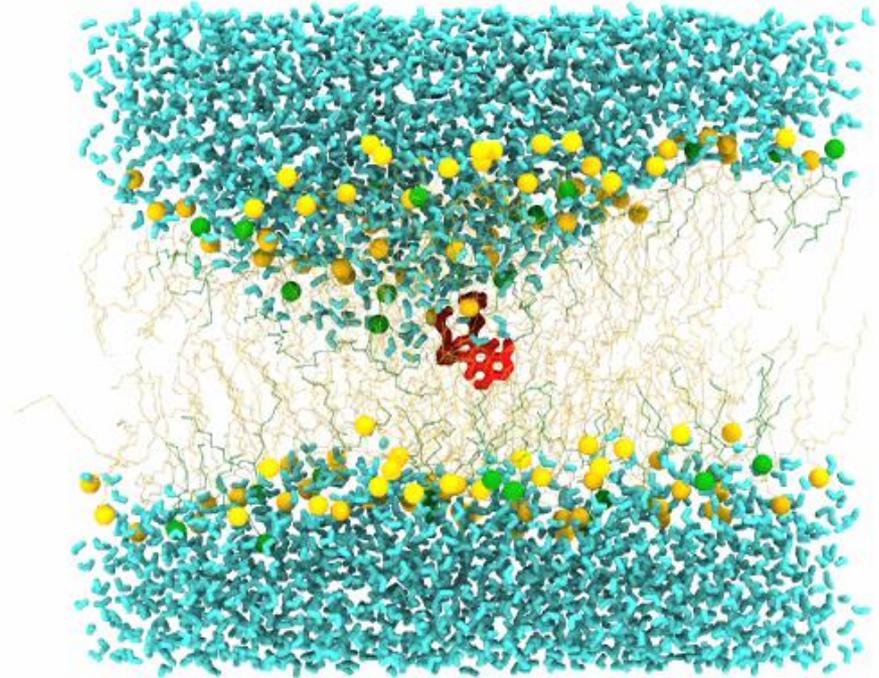


# SCS assemble transient membrane gateways

*Molecular dynamics (in silico) experiments: Mitoxantrone*



*Control*



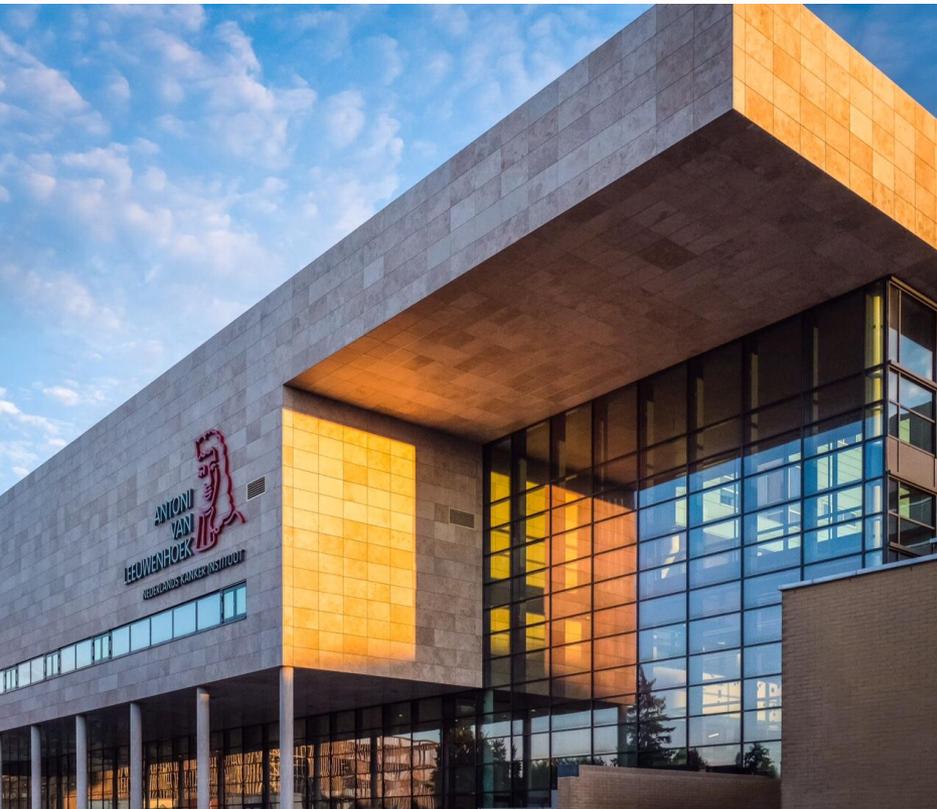
+SCS

# Summary

- Defined short-chain sphingolipids (SCS) enhance cellular doxorubicin uptake
- The SCS effect shows tumor cell preference and depends on specific lipid and drug requirements
- *In vivo* GC-Lipodox shows favorable biodistribution and overcomes drug resistance in mouse model for breast cancer
- SCS assemble transient membrane channels to facilitate drug influx
- High through-put screening identified additional agents, including mitoxantrone and PI3K inhibitors

# Future studies

- Validate hits
- Combining radiation and modified liposomal radiosensitizers
- Prepare GMP-batch for clinical phase I study (Ceronco)



# Acknowledgements



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