Thermally targeted delivery of anticancer therapeutic peptides using elastin-like biopolymers

8th International Conference and Exhibition on March 07-09, 2016 Madrid, Spain Pharmaceutics & Novel Drug Delivery Systems

Overview

- Localized tumors - current treatment
- Background of the ELP system
- Applications in cancer drug delivery
- Targeting c-Myc in breast and brain cancer

Localized Tumors - Current Treatment

- Surgical resection, followed by chemo- and/or radiotherapy
- Limited by normal tissue tolerance and/or inherent tumor radio or chemo-resistance
- Only a small fraction of the administered dose of drug reaches the tumor site, while the rest of the drug is distributed throughout the body
- To make chemotherapy more effective and less toxic, site-specific drug delivery vehicles would increase the amount of drug that reaches the intended target and would simultaneously reduce nonselective cytotoxicity.

Targeted Drug Delivery Systems

Passive Targeting

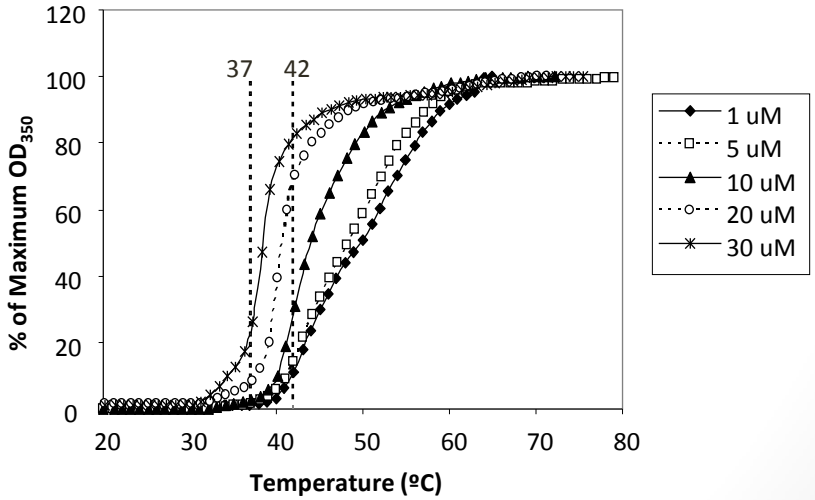
- **uses the physicochemical properties (particle diameter, hydrophilic properties, etc.) of a carrier (transporter of the drug) to control behavior inside the body**

Active Targeting

- **adds special mechanisms to the passive type to tightly control the directionality toward the target tissue.**
- **"missile drugs" that use carriers consisting of combinations of ligands, e.g. antibodies, peptides, sugar chains, etc., that have specific molecular recognition features that can find target molecules of certain cells that make up the target tissue**

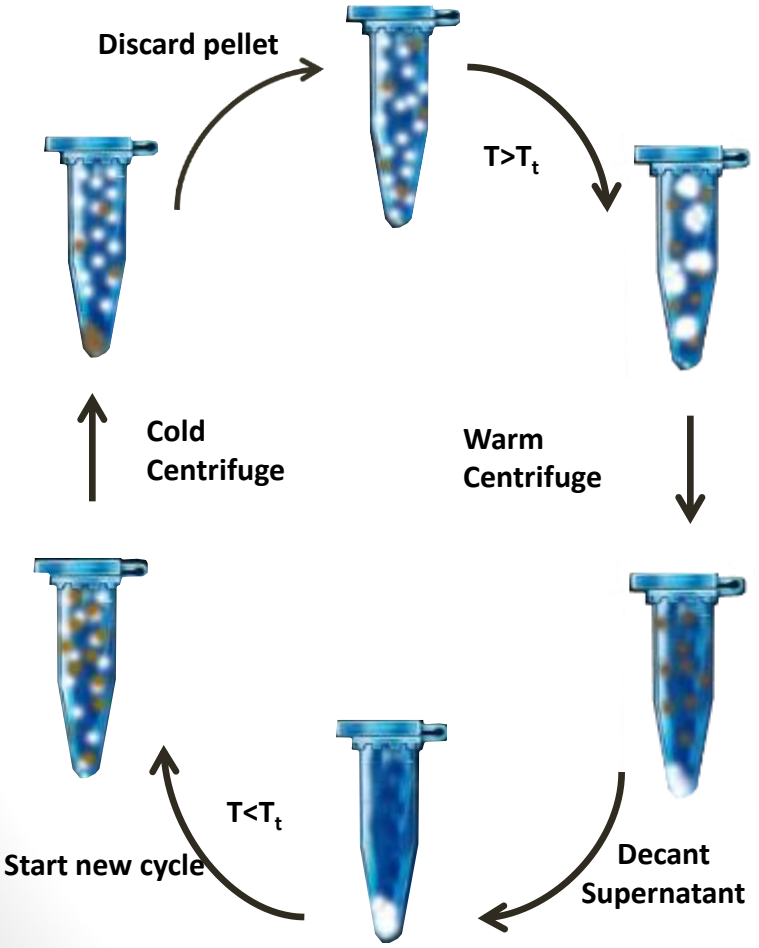
Elastin-like Polypeptide (ELP)

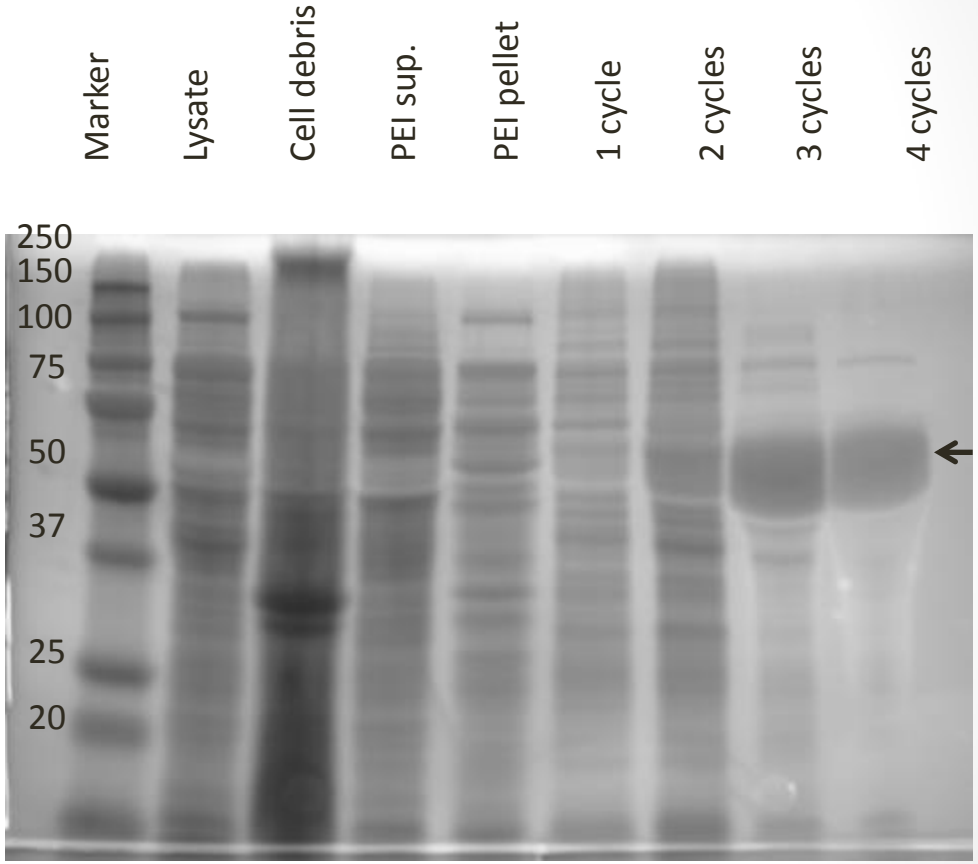
- Synthetic protein consisting of VPGxG repeats
- Thermally responsive
- Biologically inert
- Expressed and purified from *E. coli*
- Can be fused to therapeutic peptides or small molecule drugs

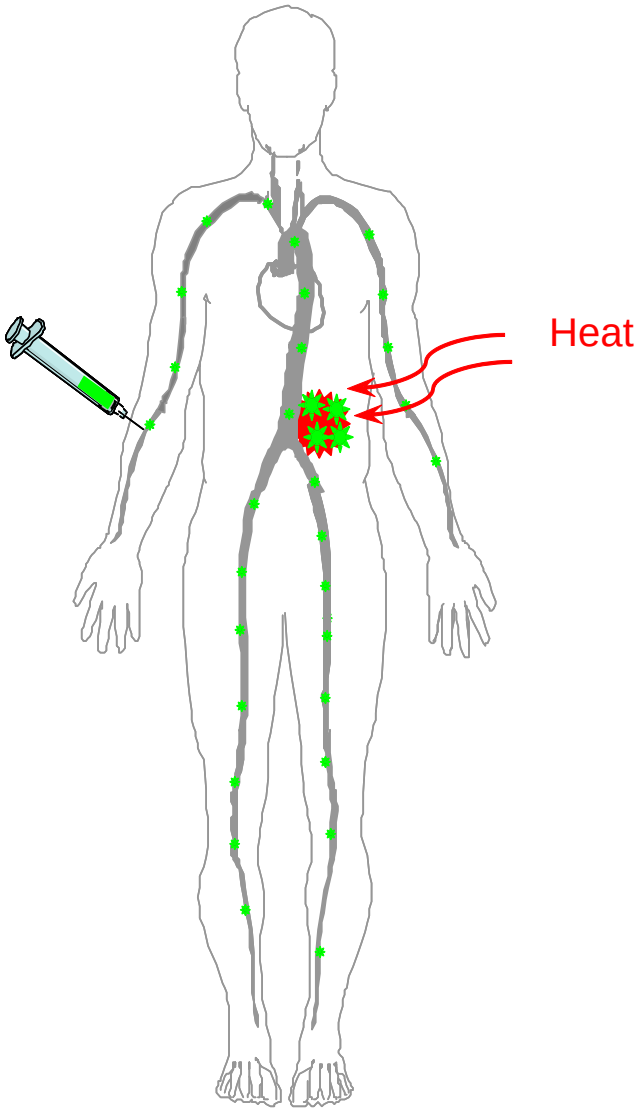


Urry, D.W. *Prog. Biophys. Molec. Biol.* 57:23-57 (1992).

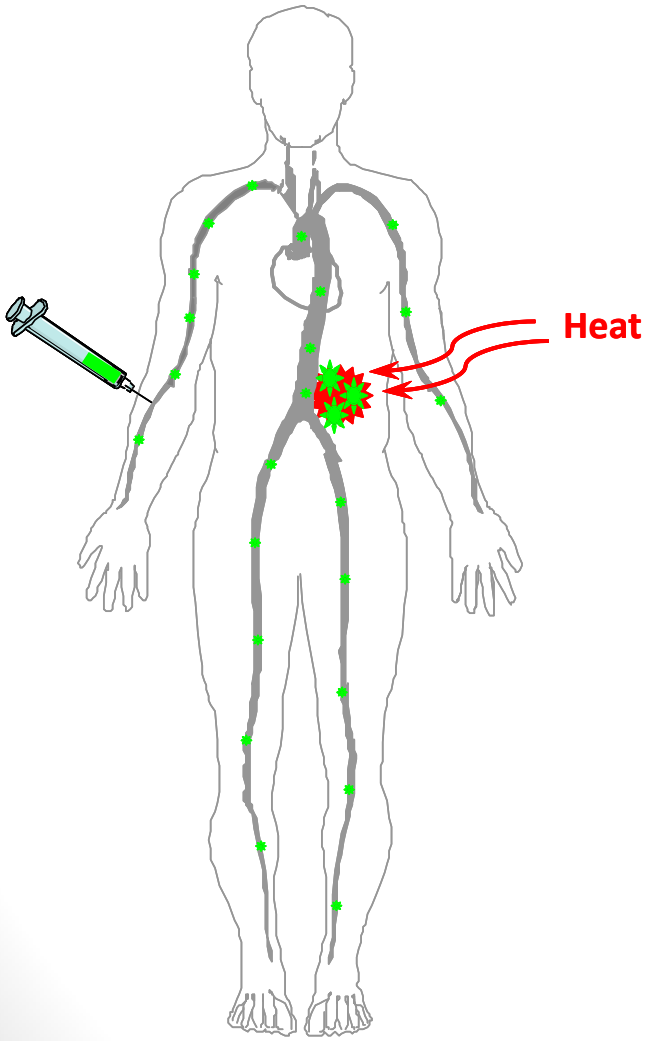
ELP Expression and Purification






Heat

ELP as a Drug Delivery Vector



- Advantages of Polymeric Drug Carriers:
  - Passive targeting: Enhanced permeability and retention effect.
  - Increased solubility and plasma half-life.
  - Systemic toxicity is reduced.
- Further advantages of ELP:
  - Active Targeting: Thermally responsive.
  - Genetically engineered, easy to manipulate sequence, express in *E. coli*, and purify.
- Hypothesis: Systemically injected ELP will accumulate at locally heated regions, but will circulate and eventually be cleared in non-heated tissues.
- Hyperthermia can be applied in the clinical setting using high intensity focused ultrasound or radio-frequency radiation.

Thermal Targeting

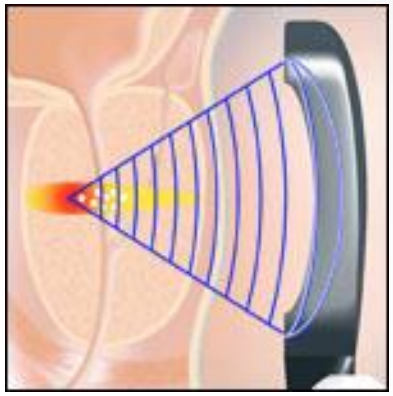
T_{body} (37-38 ˚C) < T_t (~41 ˚C) < $T_{hyperthermia}$ (42-43 ˚C)

Complimentary with established advantages of:

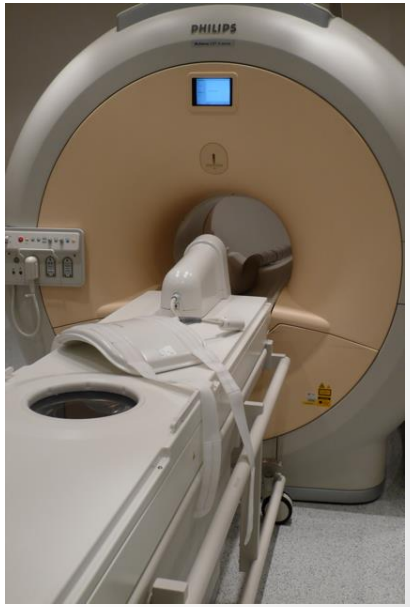
- Macromolecular Carriers
  - Increased solubility
  - Increased plasma half-life
  - High drug loading capacity
- Hyperthermia
  - Increased chemo- and radiosensitivity
  - Increased macromolecular extravasation

Clinical Application of Hyperthermia

- HIFU: High Intensity Focused Ultrasound
  - HIFU technology uses a high-intensity convergent ultrasound beam generated by high power transducers to produce heat.
  - As an acoustic wave propagates through the tissue, part of it is absorbed and converted to heat. With focused beams, a very small focus can be achieved deep in tissues.



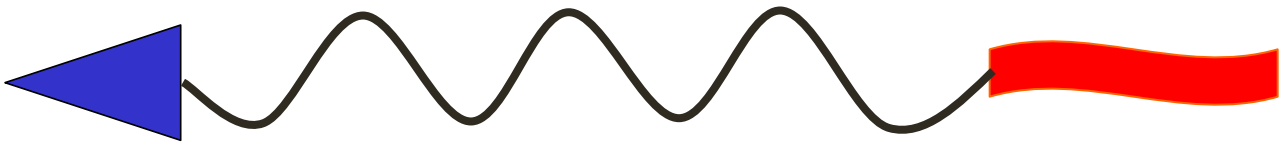
MRI-guided HIFU



Therapeutic Peptides

- Advantages:
  - Easy to design using “rational” strategies
  - Can target nearly any protein or pathway
  - Highly specific for their targets
- Disadvantages:
  - Short plasma half-life
  - Easily degraded *in vivo*
  - Don’t penetrate biological membranes
  - Can be immunogenic

Design of the ELP Drug Delivery Vector



Cell Penetrating Peptide

ELP

Drug or inhibitory peptide

CPP	Sequence
Penetratin	RQIKIWFQNRRMKWKK
Tat	YGRKKRRQRRR
Bac	RRIRPRPPRLPRPRPRPLPFPRPG
SynB1	RGGRLSYSRRRFSTSTGR

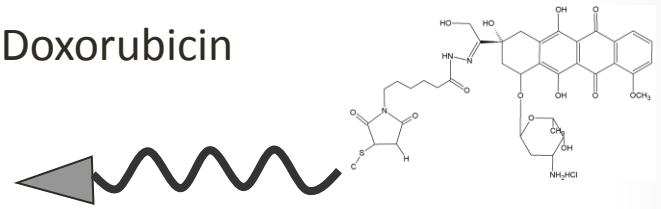
Previous Applications of ELP for Drug Delivery in Cancer

Therapeutic Peptides

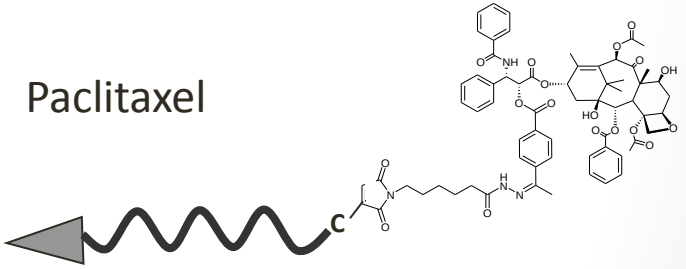
Protein Target	Peptide Name	Sequence
c-Myc	H1-S6A, F8A	NELKRAFAALRDQI
p21	W10	GRKRRQTSMTDFYHSKRRLIFSKRKP
IKKβ	NBD	TALDWSWLQTE
p53	Peptide 46	GSRAHSSHLKSKKGQSTSRHKK
PRMT5	GRG	GRGGRGGRGGRGGRGGRGRG
Bad	BH3 Bad	NLWAAQRYGRELRRMSDEFVD
(mitochondrial membrane)	KLAK	KLAKLAKKLAKLAK

Small Molecule Drugs

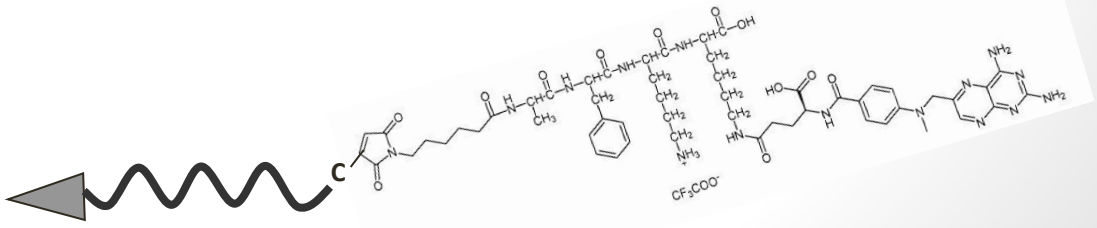
Doxorubicin



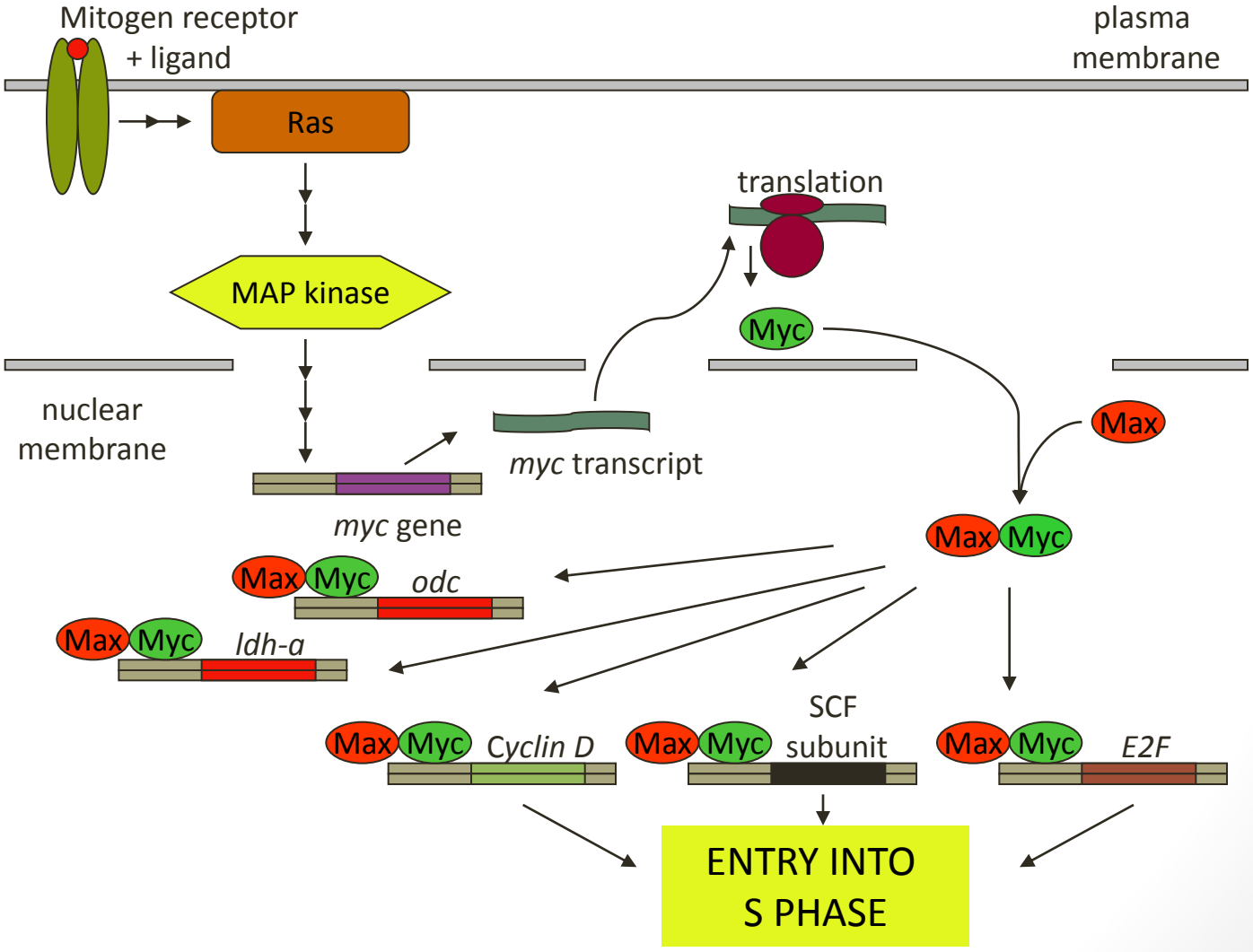
Paclitaxel



Methotrexate



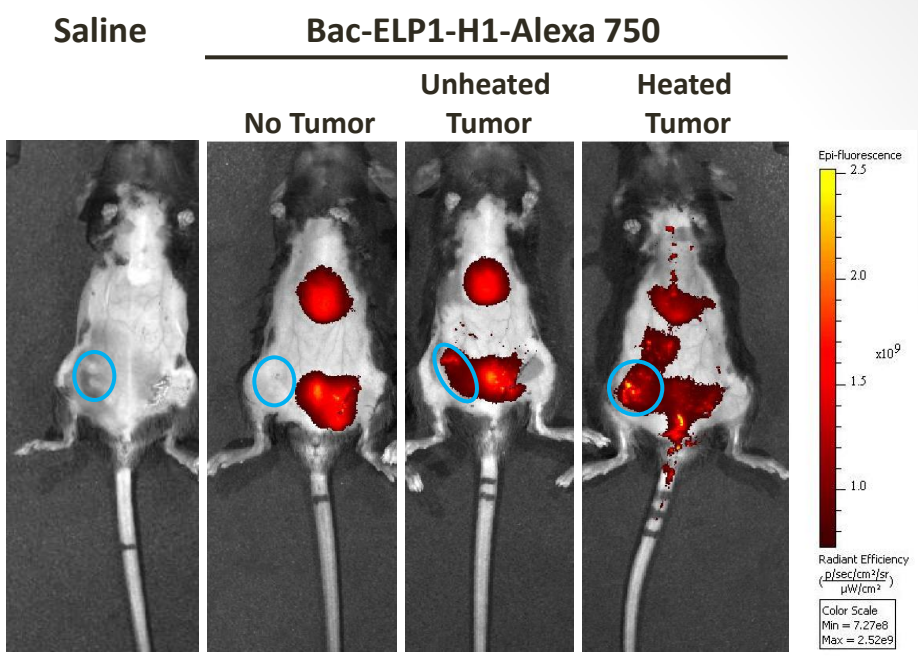
A c-Myc Inhibitory Peptide

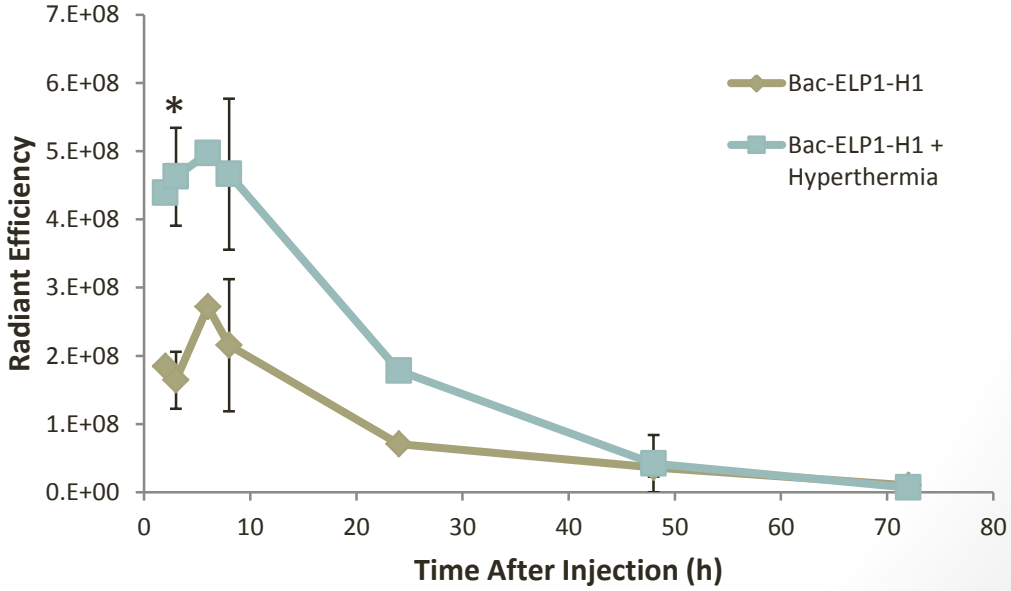


In vivo Evaluation - E0771 Breast Cancer Model

- Medullary adenocarcinoma
- Isolated from a spontaneous tumor in C57BL/6 mice
- Very aggressive, and will invade the peritoneal cavity and metastasize to the lungs

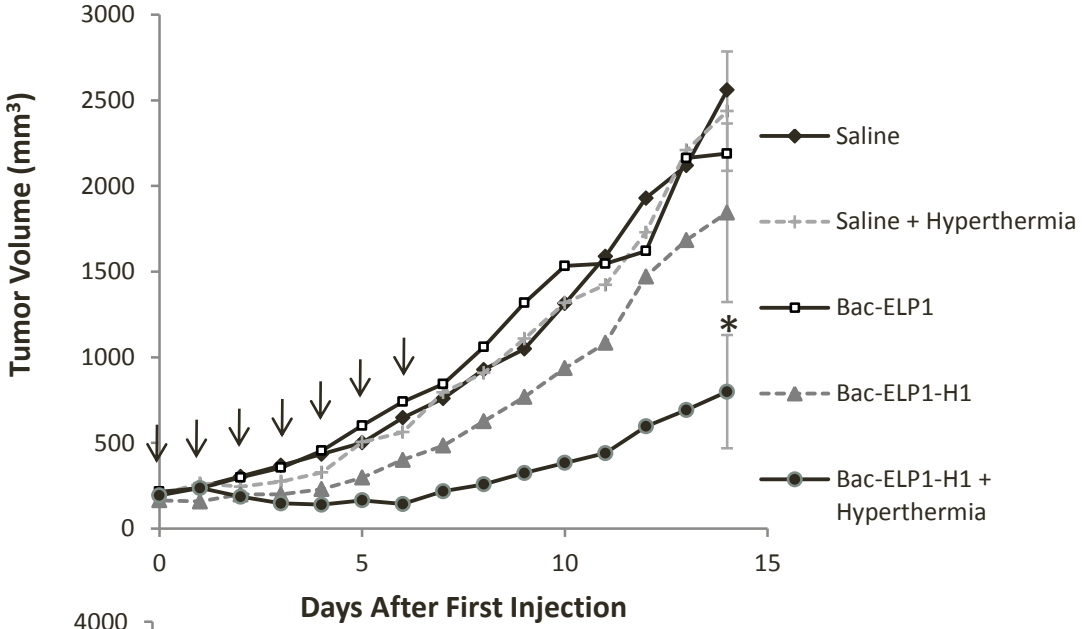
Bac-ELP-H1 Tumor Uptake

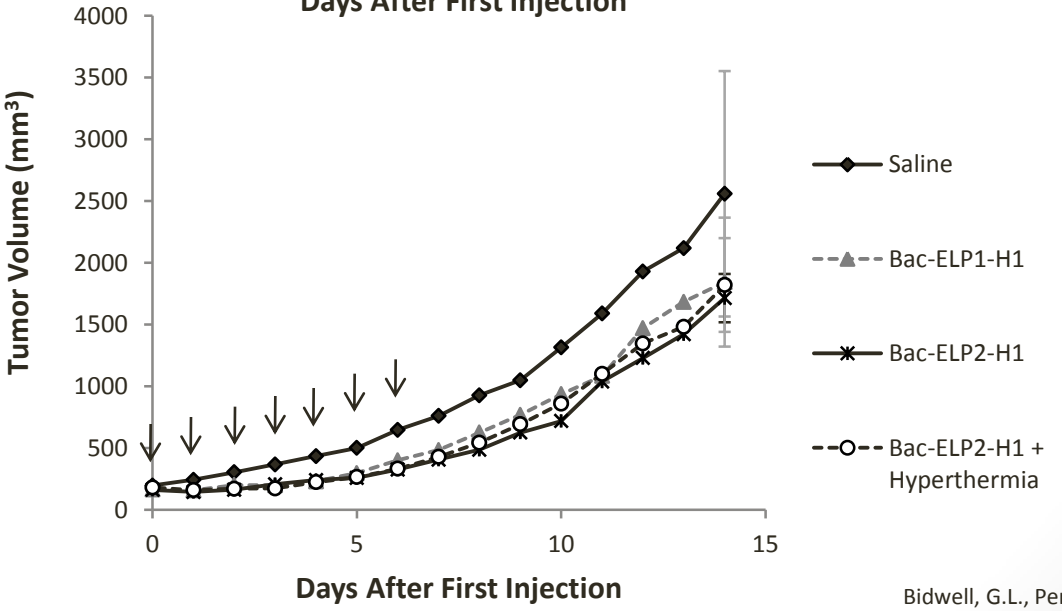




Bidwell, G.L., Perkins, E., and Raucher, D. *Cancer Letters.* 2012.

Reduction of E0771 Breast Tumors

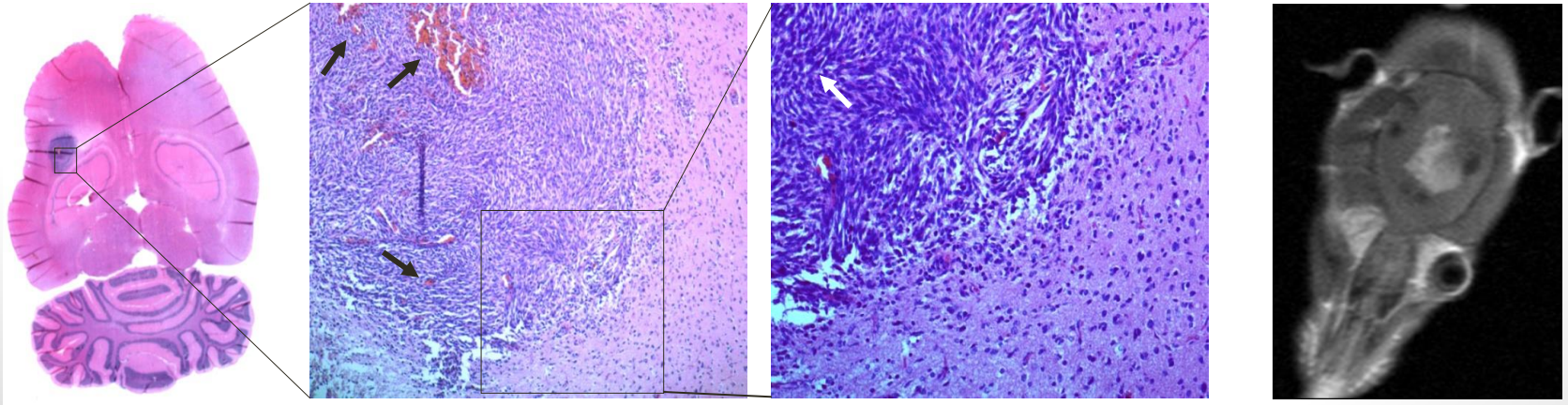




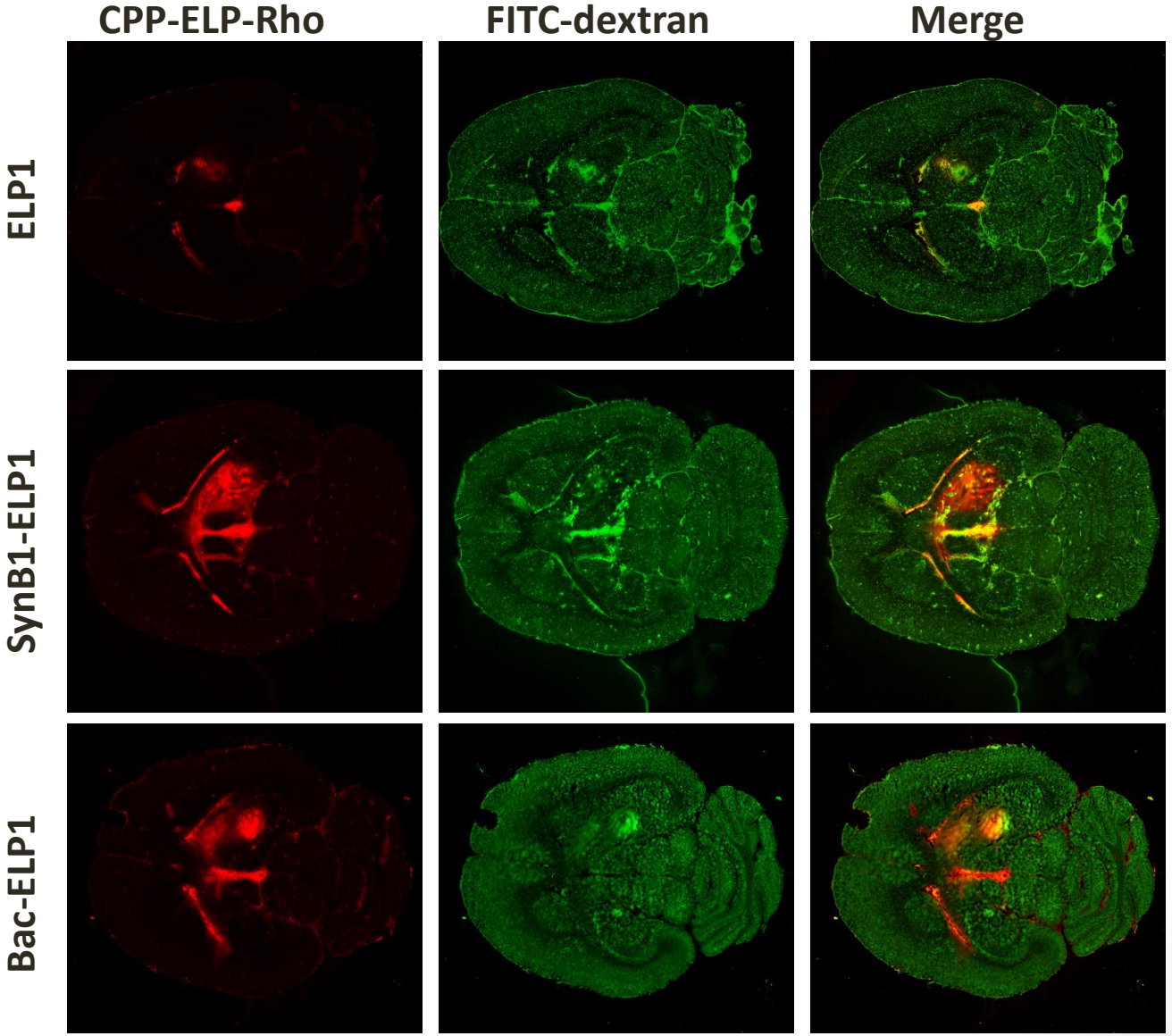
Bidwell, G.L., Perkins, E., and Raucher, D. *Cancer Letters.* 2012.

The C6 Glioma Model

- C6 is a rat glioma model
- C6 cells were derived from methylnitrosourea-induced gliomas in Wistar rats
- C6 cells generate rapidly growing tumors when injected orthotopically in SD rats
- They mimic human glioblastoma in histology, rapid proliferation, and intracranial dissemination.



Delivery to Intracranial C6 Tumors



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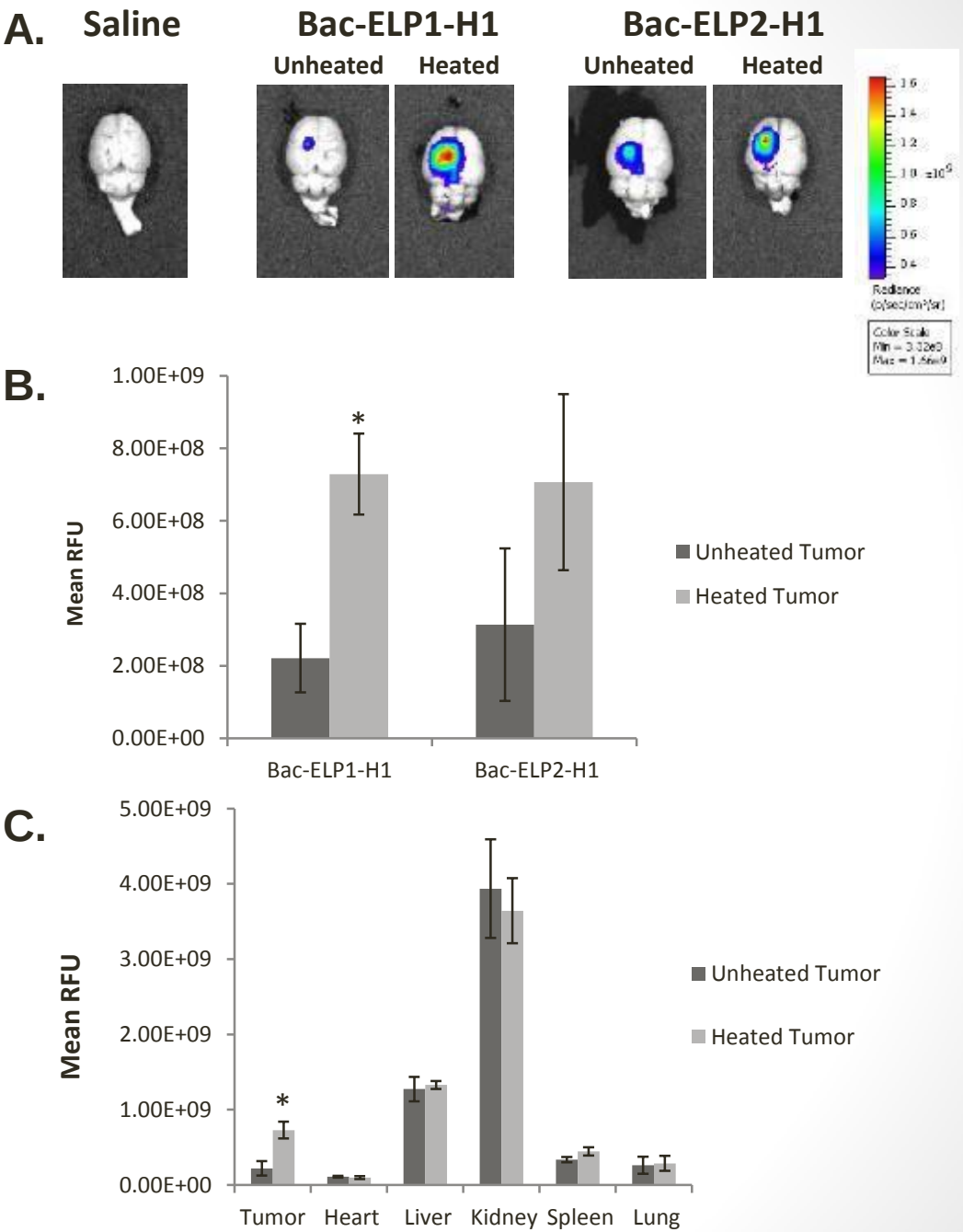
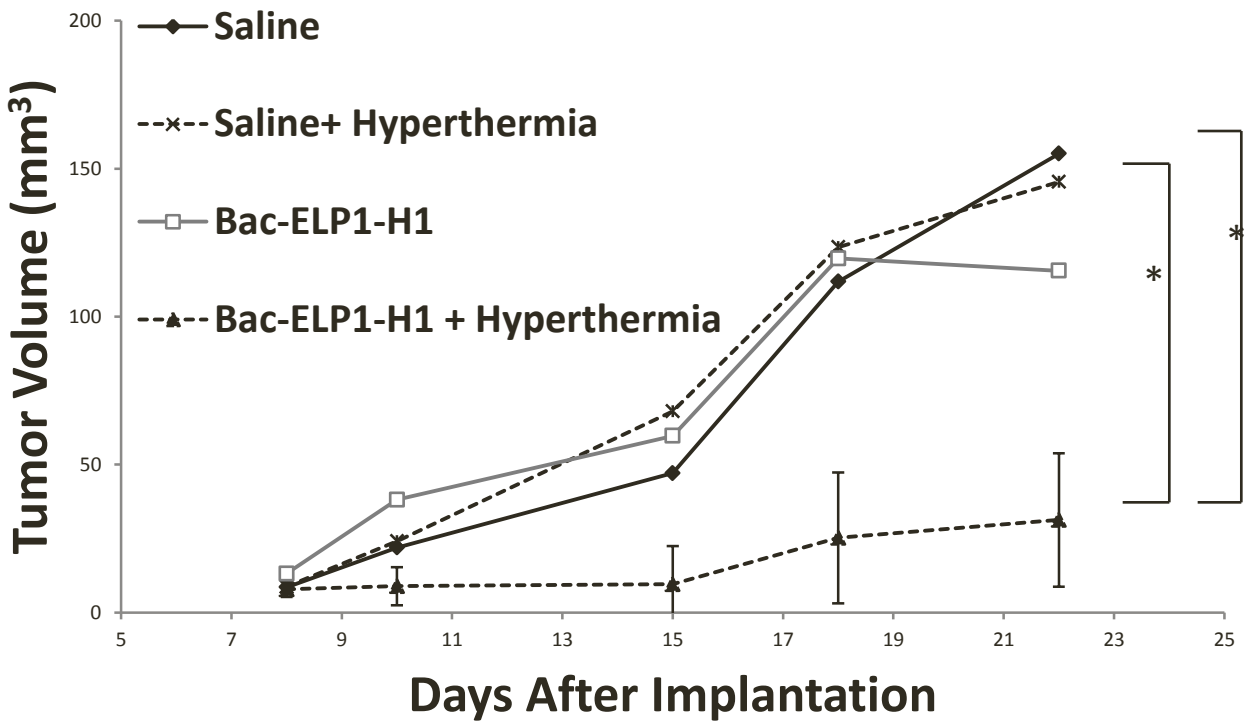
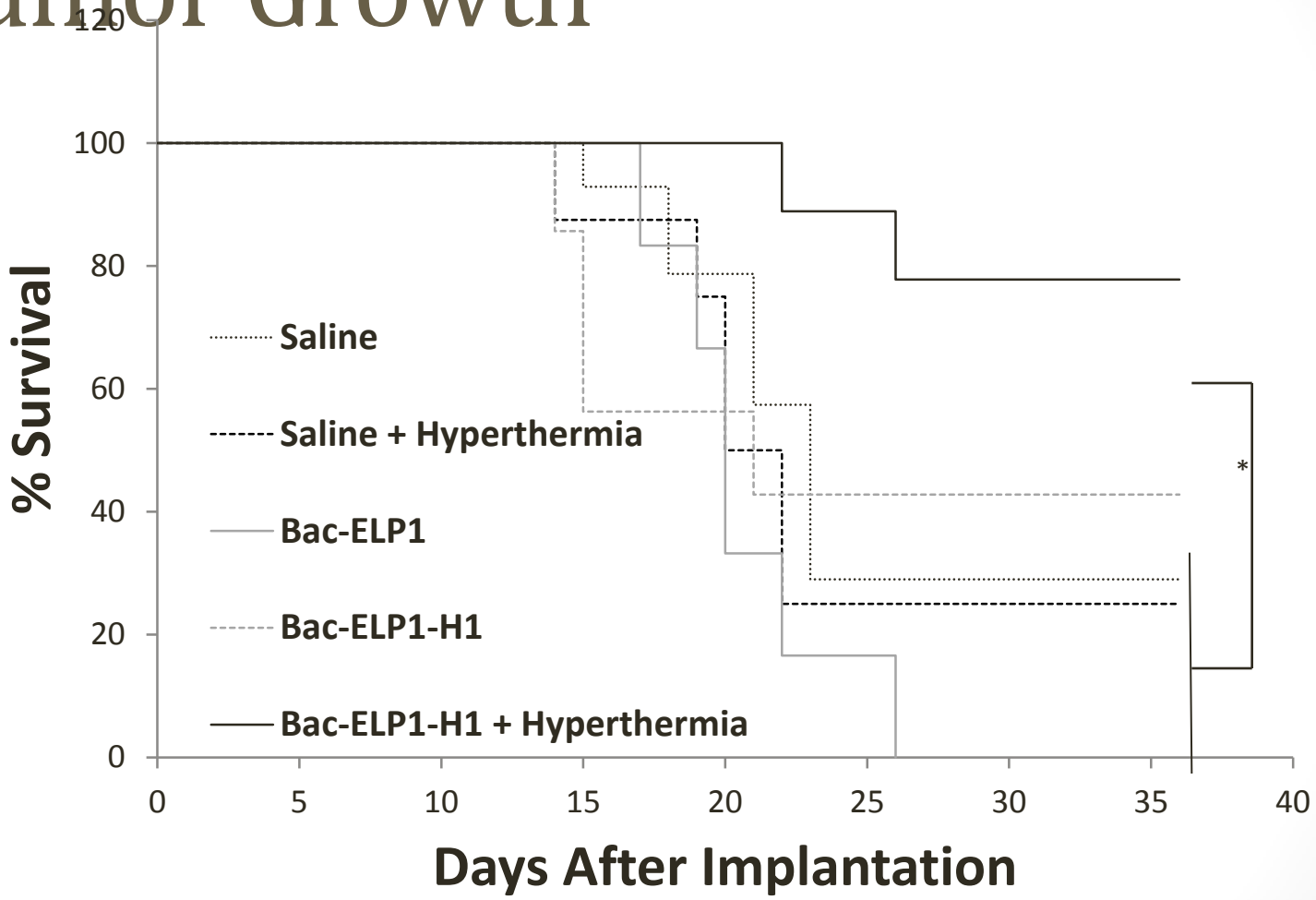


Inhibition of Intracranial C6 Tumor Growth

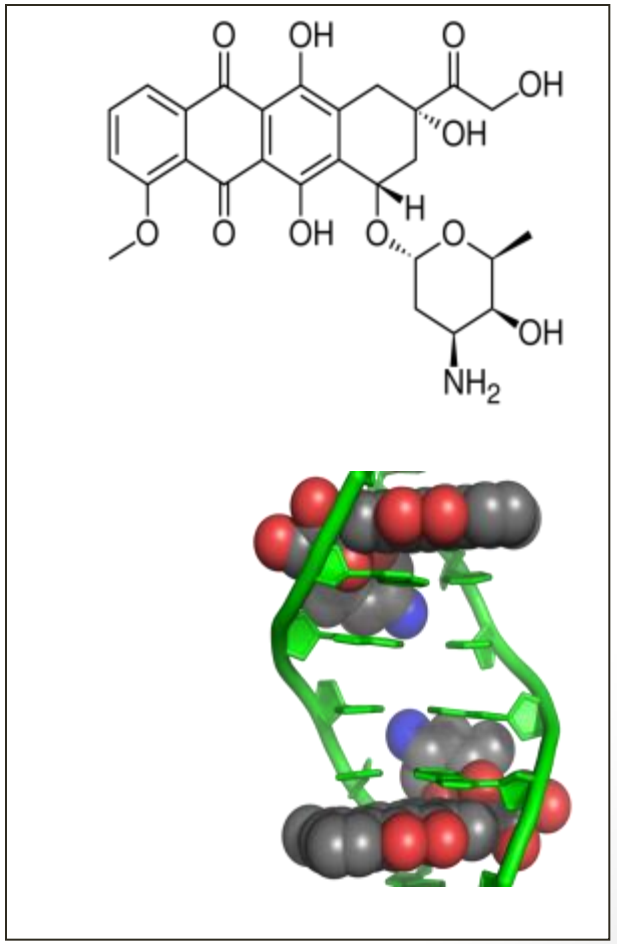


Inhibition of Intracranial C6 Tumor Growth

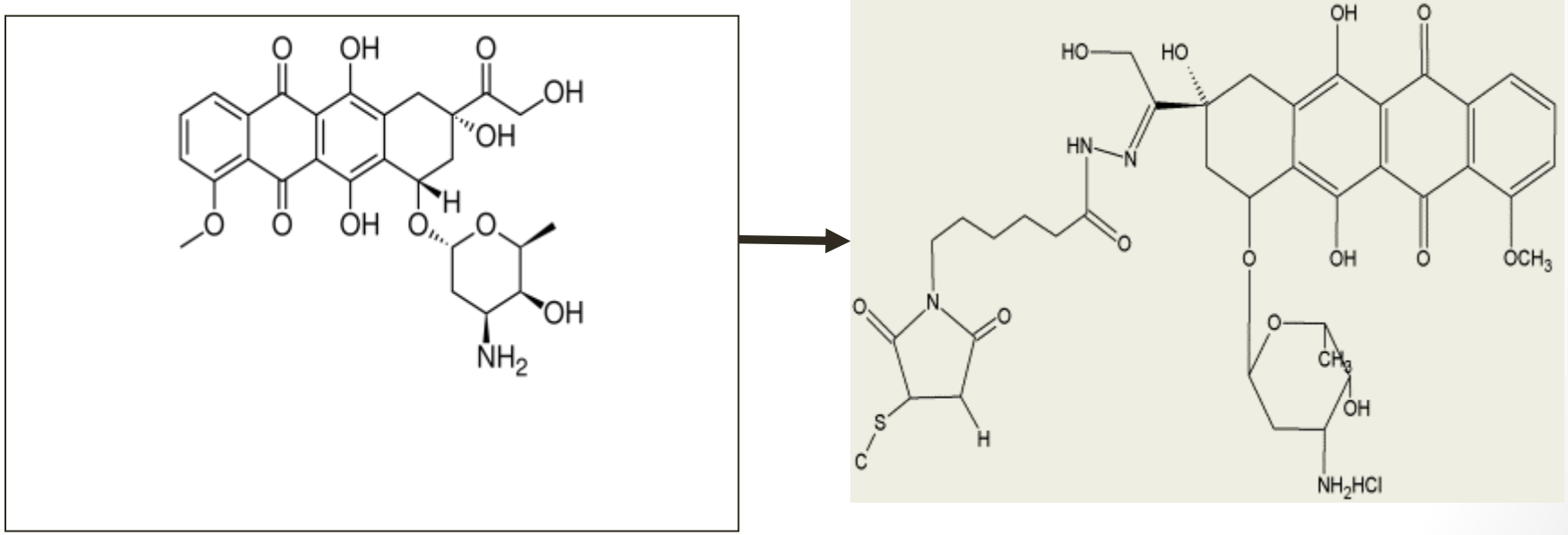


Delivery of Doxorubicin (Dox) with ELP

- Doxorubicin is commonly used in the treatment of a wide range of cancers, including hematological malignancies, many types of carcinoma, and soft tissue sarcomas.
- Intercalates into DNA and induces double strand breaks by stabilizing topoisomerase II cleavage complexes.
- Acute adverse effects of doxorubicin can include nausea, vomiting, and heart arrhythmias.
- Doxorubicin's most serious adverse effect is life-threatening heart damage.



Delivery of Doxorubicin (Dox) with ELP



Dr. Kratz, CytRx Corporation,
Freiburg, Germany

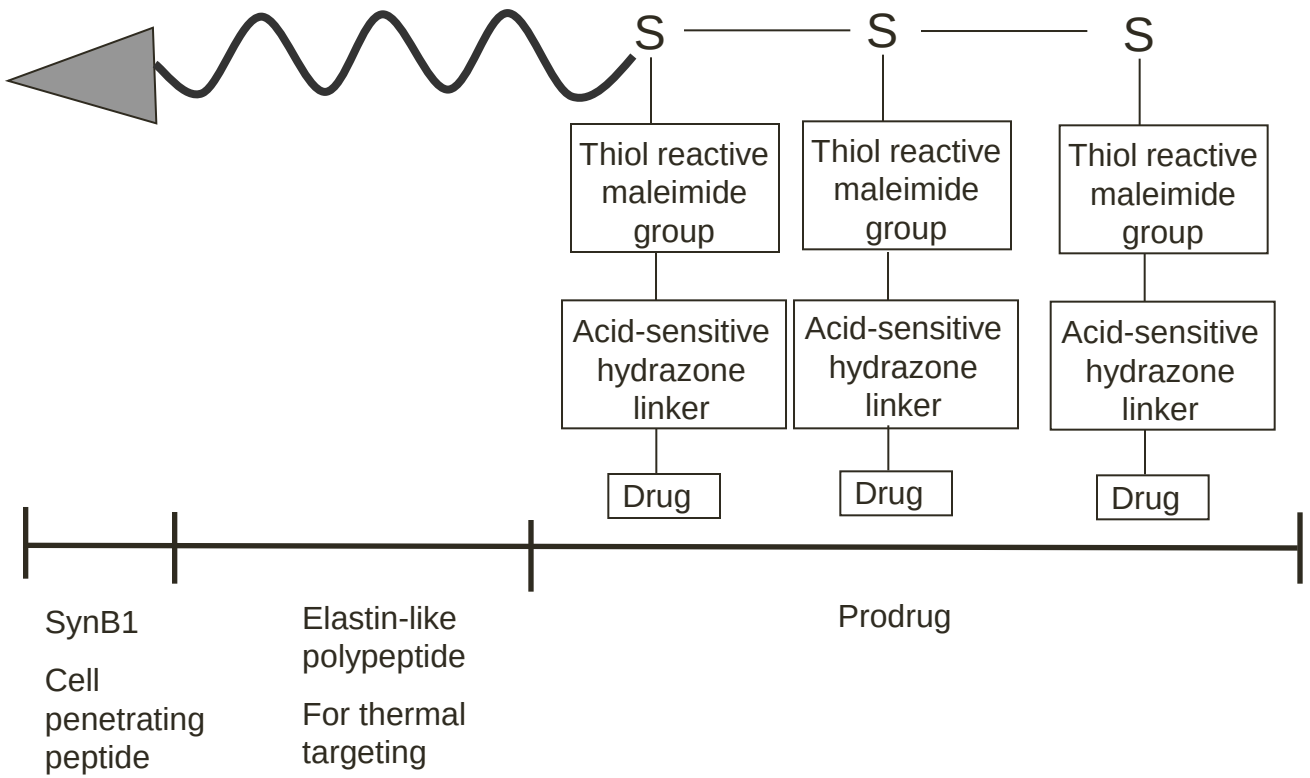
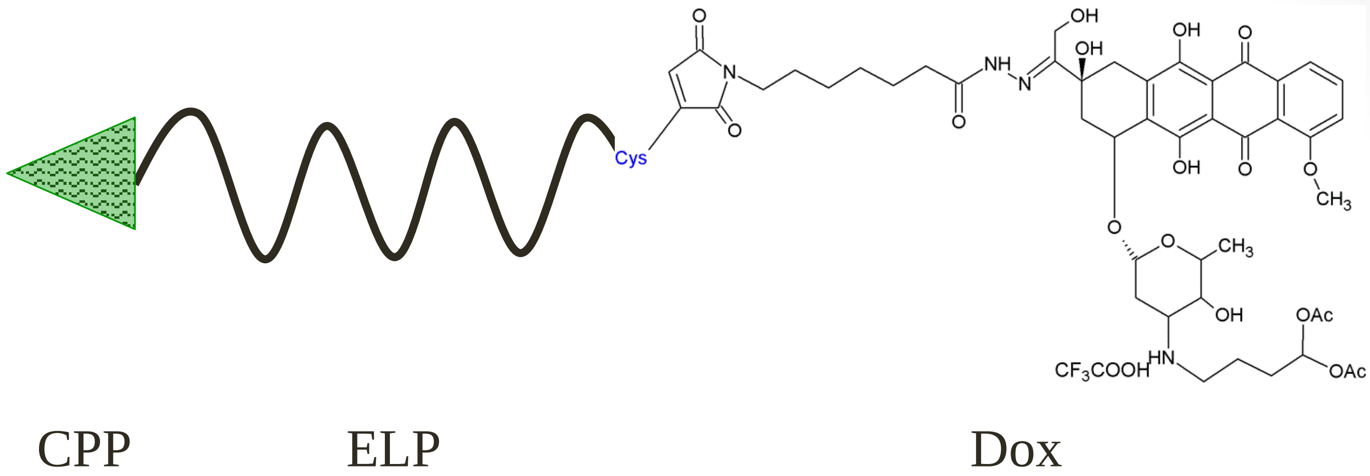


Figure 1. Schematics of the ELP-based drug delivery vector. The delivery system consists of the SynB1 cell penetrating peptide at the N-terminus, followed by the thermally responsive elastin-like polypeptide with three terminal cysteine residues, and the thiol reactive prodrugs of doxorubicin, paclitaxel or methotrexate,


Cys
O
N
NH
N
OH
OH
OH
O
OH
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O
CH3
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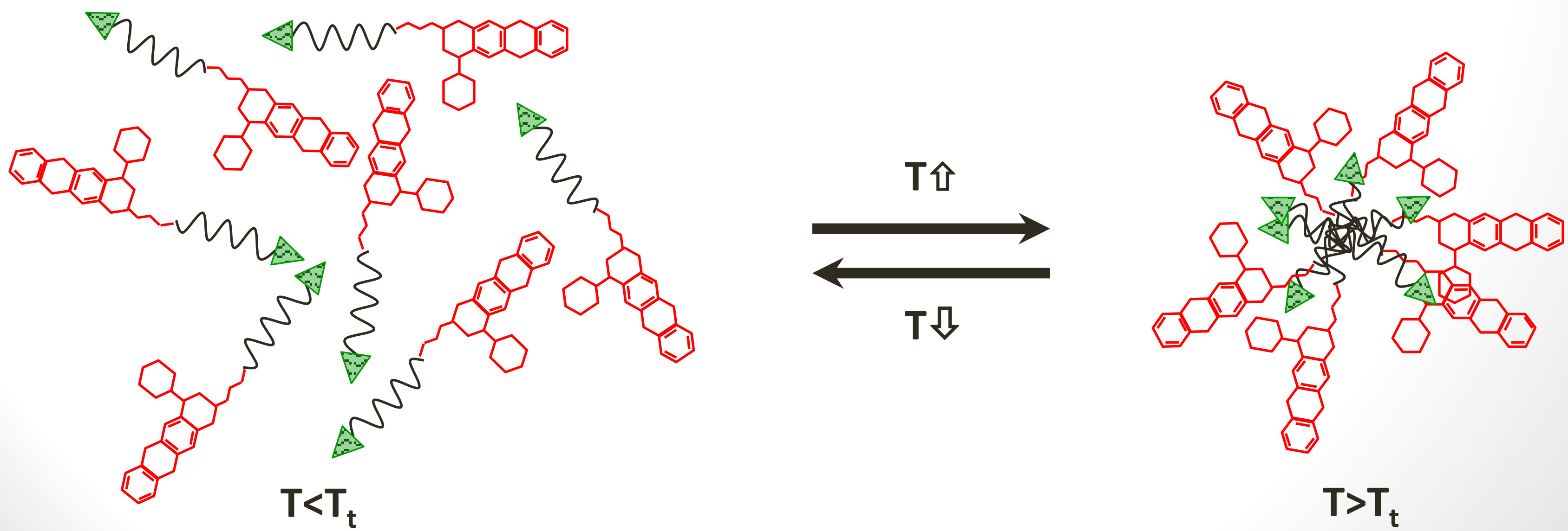
O
CH3
OH
OAc
CF3COOH
HN
OAc
CPP
ELP
Dox


T⇧
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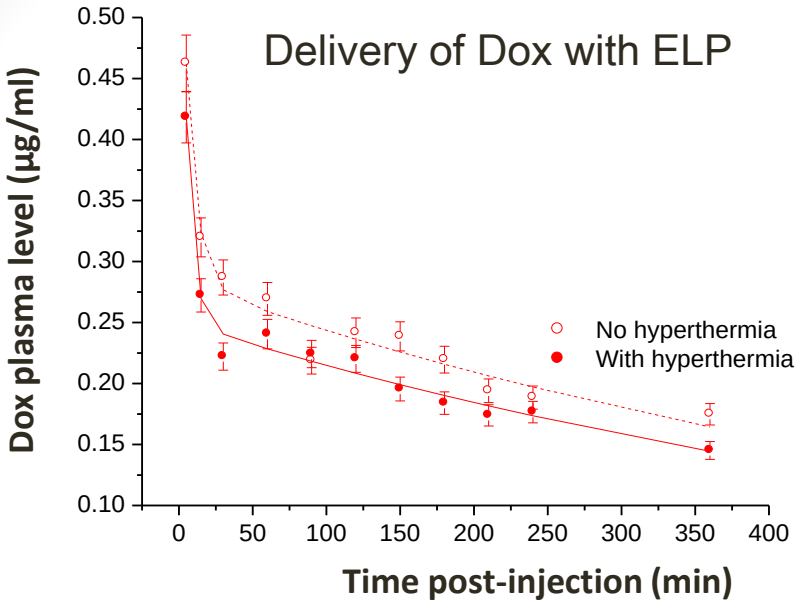
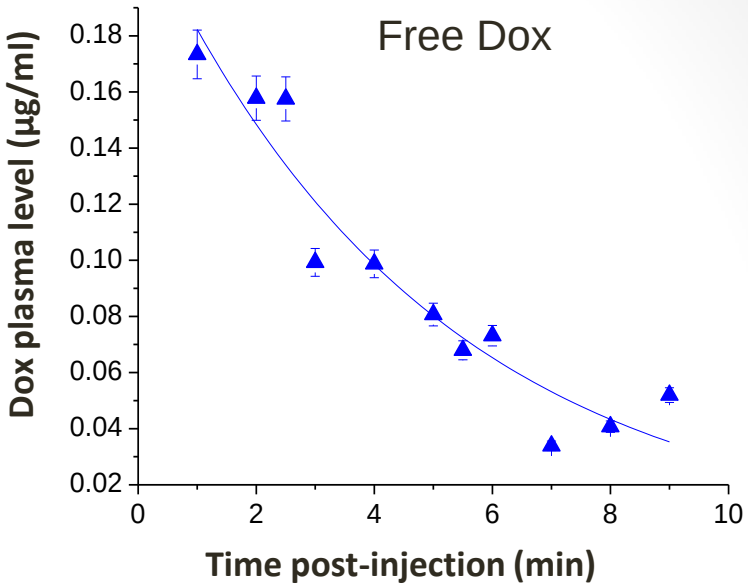
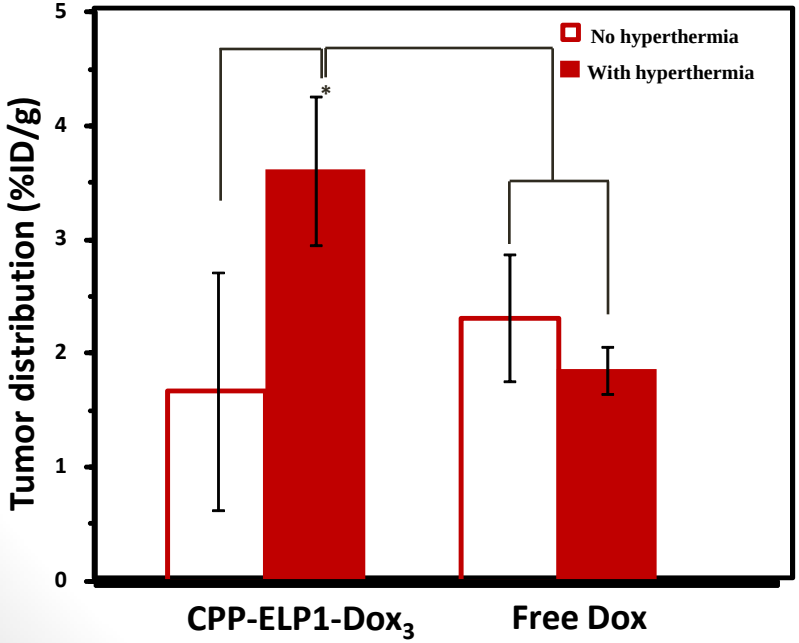
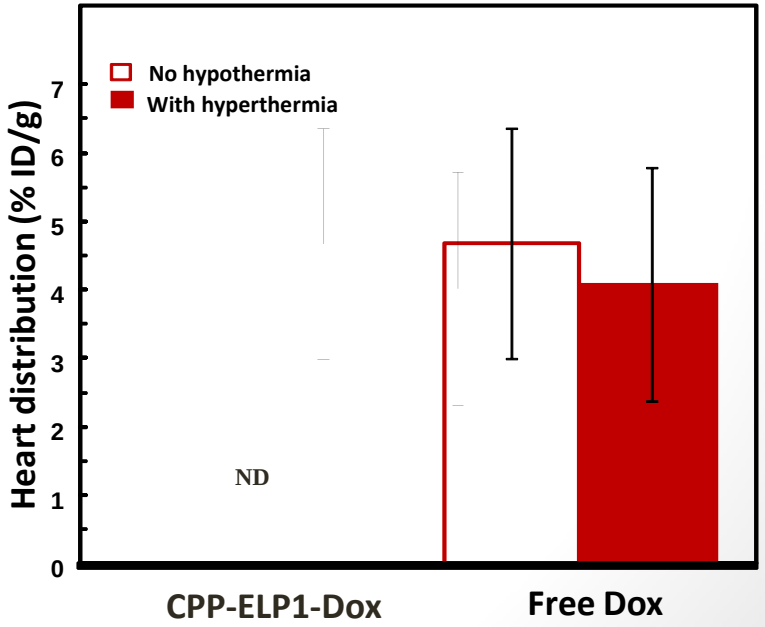
Plasma Clearance and Heart Toxicity

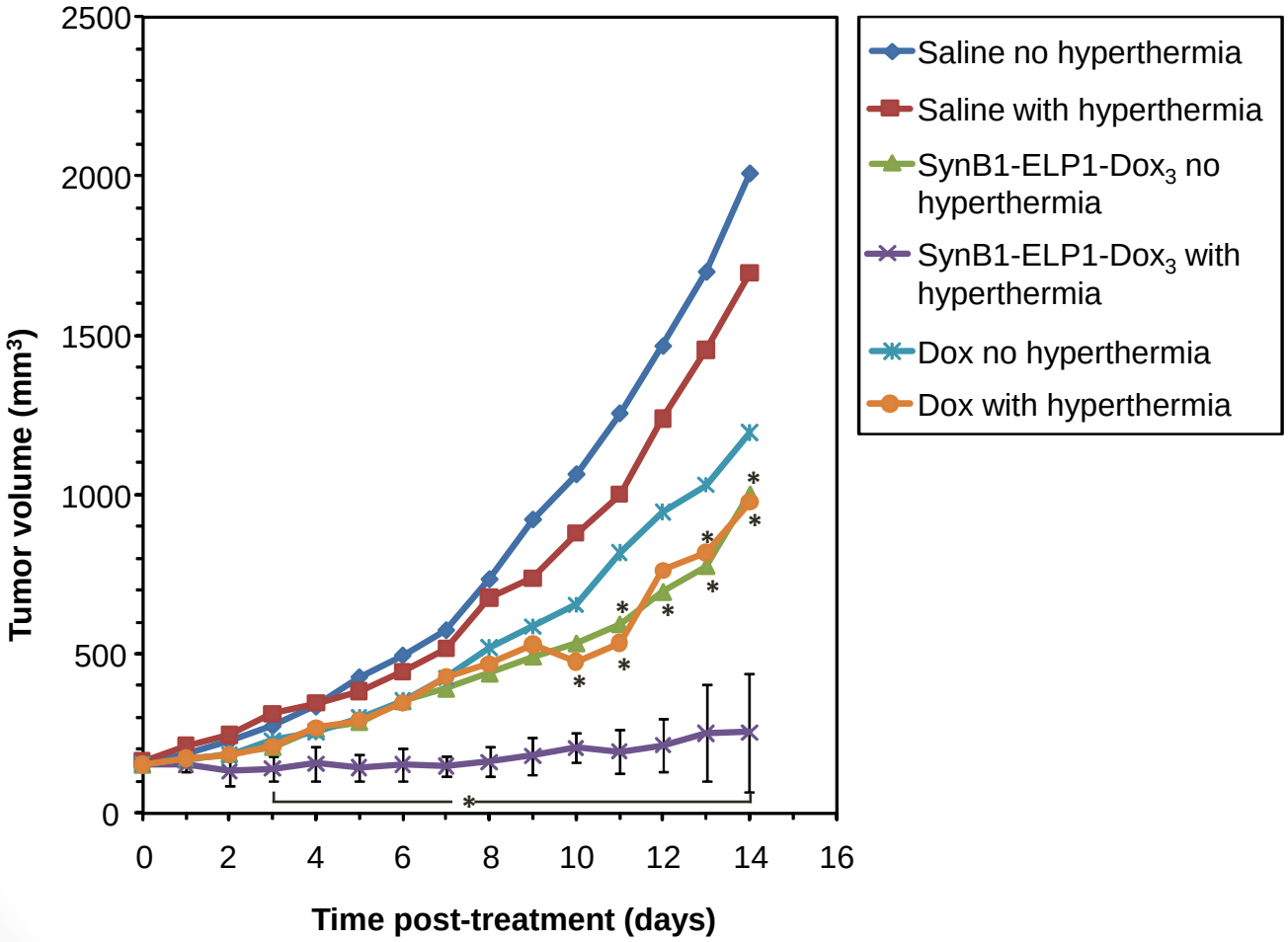




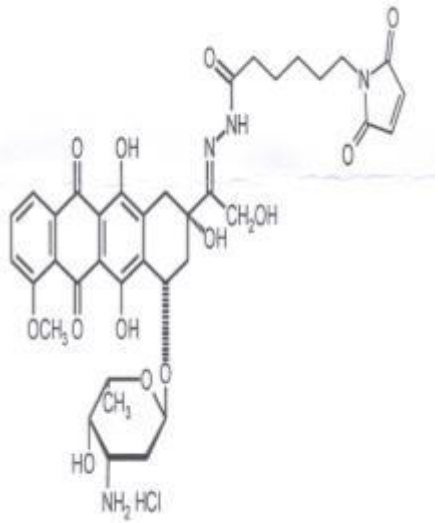




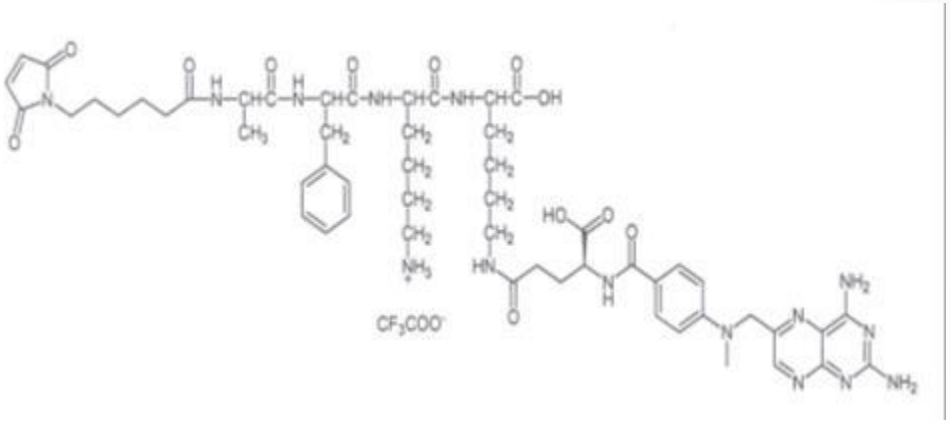
E0771 Tumor Reduction with SynB1-ELP1-ggc3-Dox injected on Day 0, Day2, and Day 4



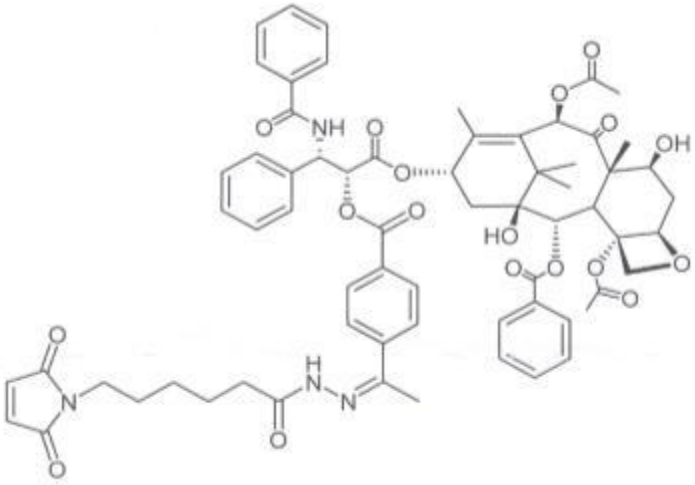
Thermally targeted delivery of chemotherapeutics (Dr. Kratz)



Doxorubicin-EMC



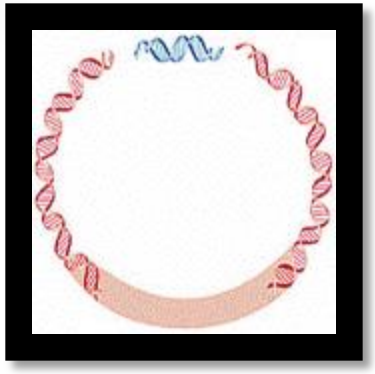
Methotrexate-EMC



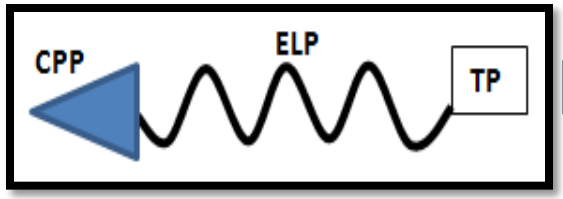
Paclitaxel-EMC

1.Moktan, S., Ryppa, C., Kratz, F., Raucher D.. A thermally responsive biopolymer conjugated to an acid sensitive derivative of paclitaxel stabilizes microtubules, arrests cell cycle, and induces apoptosis. *Investigational New Drugs, in press.*

SUMMARY



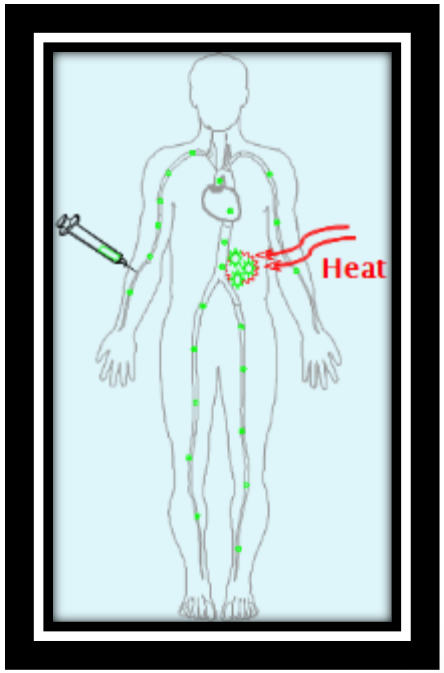
Molecular Biology



Protein on Drug

Tissue Culture

Animal Treatment



Human Treatment

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  - Dr. Judy James
  - Dr. Jack Correia
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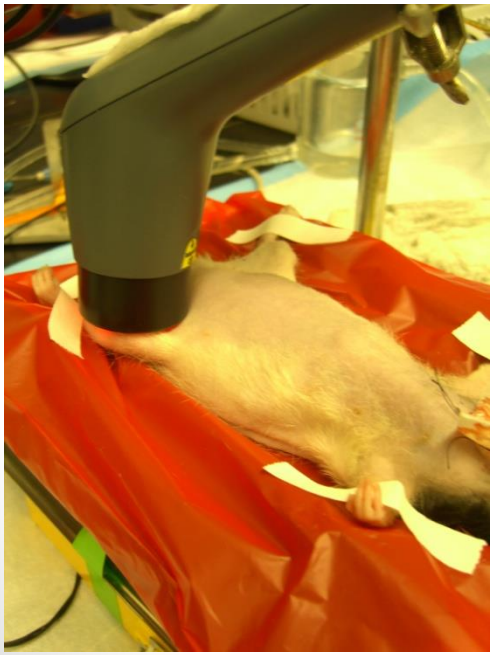
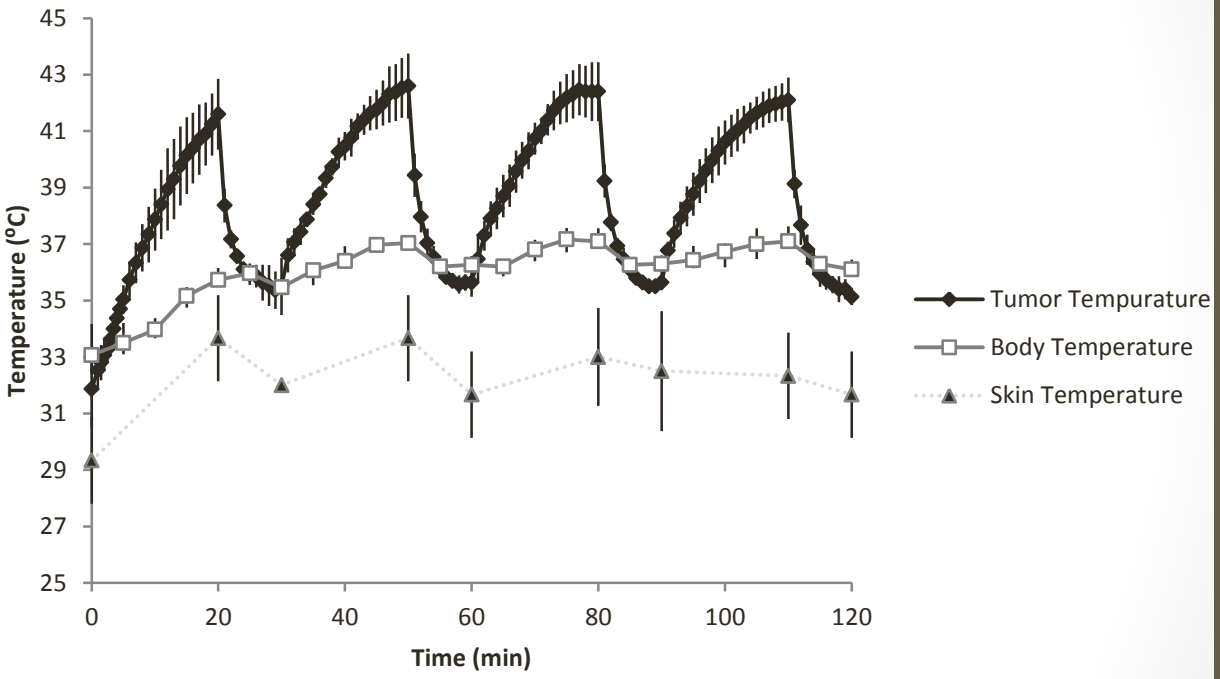
  - Dr. Ana Levenson
  - Dr. Felix Kratz
  - Dr. Luis Martinez
  - Dr. Lucio Miele
  - Dr. Guri Tzivion
  - Dr. Parminder Vig

Future Studies and Collaborations

- Delivery of chemotherapeutics
- Targeting Ras Signaling Cascade
- Thermally targeted therapy for prostate cancer
- Targeting mutant p53 protein to increase selectivity for chemotherapeutics
- Targeting Notch signaling pathway
- Targeting splicing machinery components with therapeutic peptides
- Biophysical characterization of an Elastin-Like-Polypeptide
- Application of ELP in treatment of SCA-1

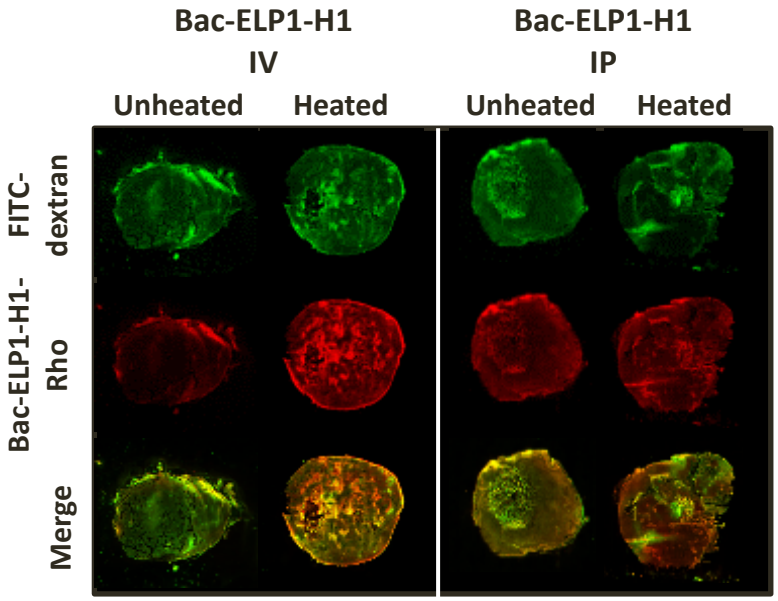
Tumor Heating with IR Light

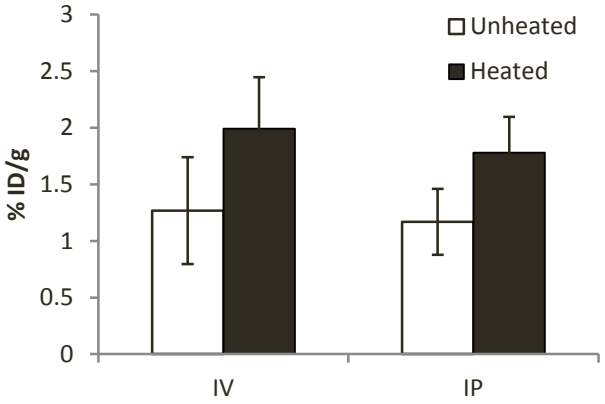


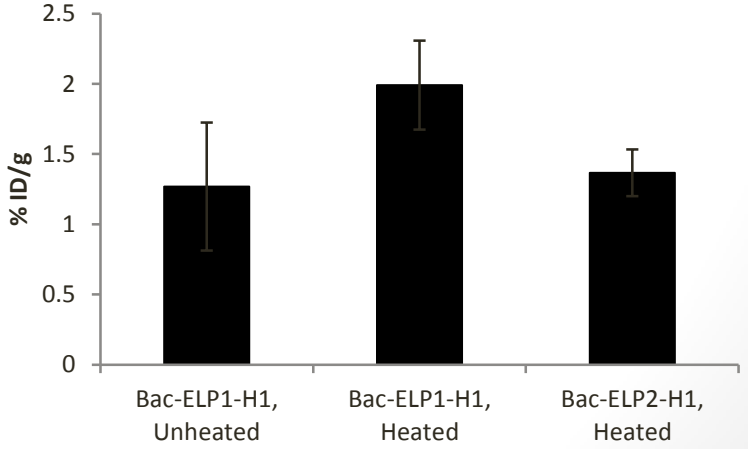


Bidwell, G.L., Perkins, E., and Raucher, D. *Cancer Letters.* 2012.

Bac-ELP-H1 Tumor Uptake







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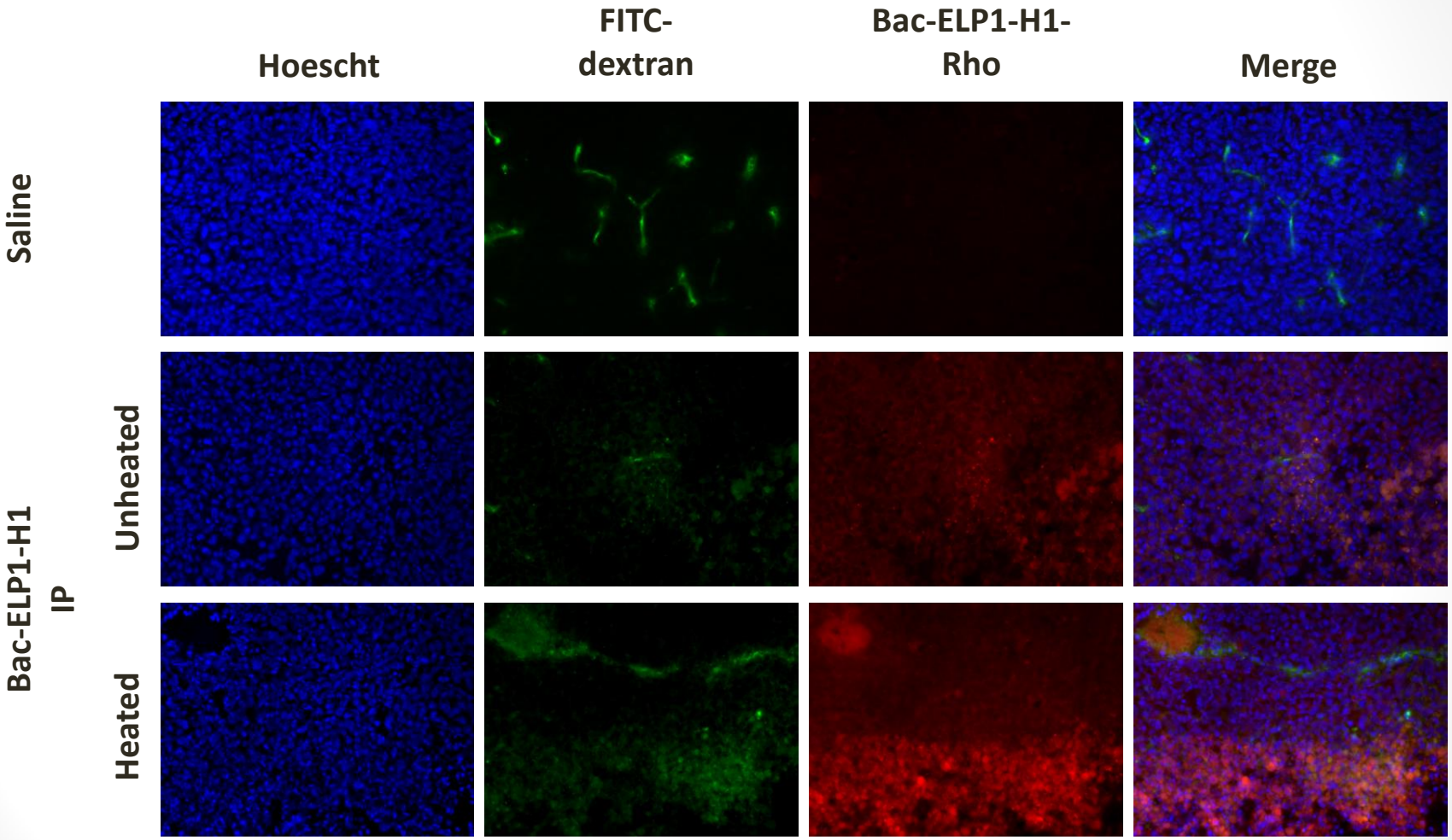

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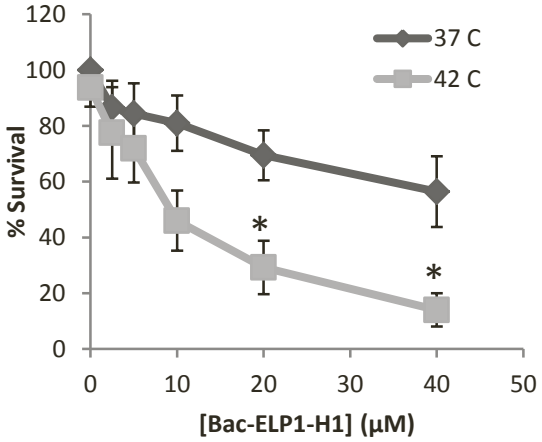
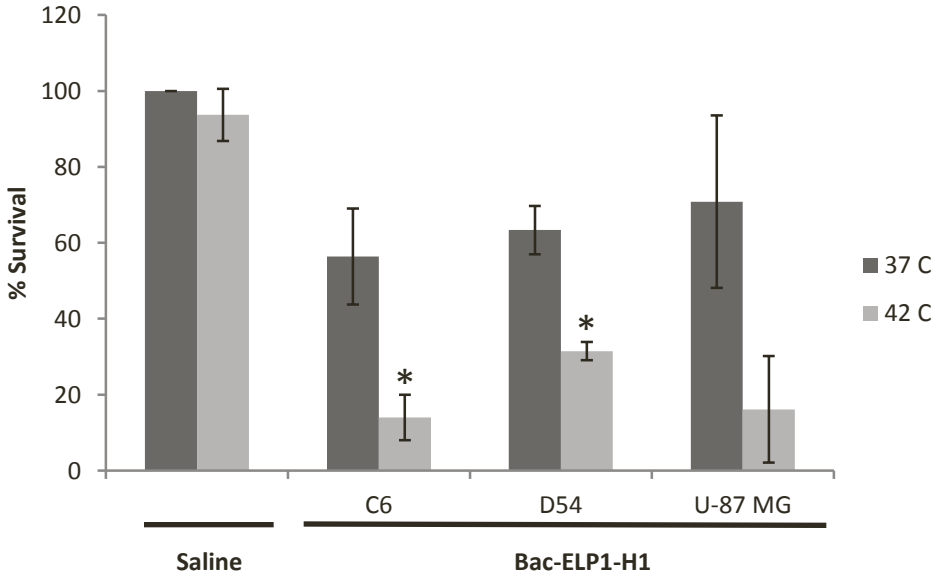
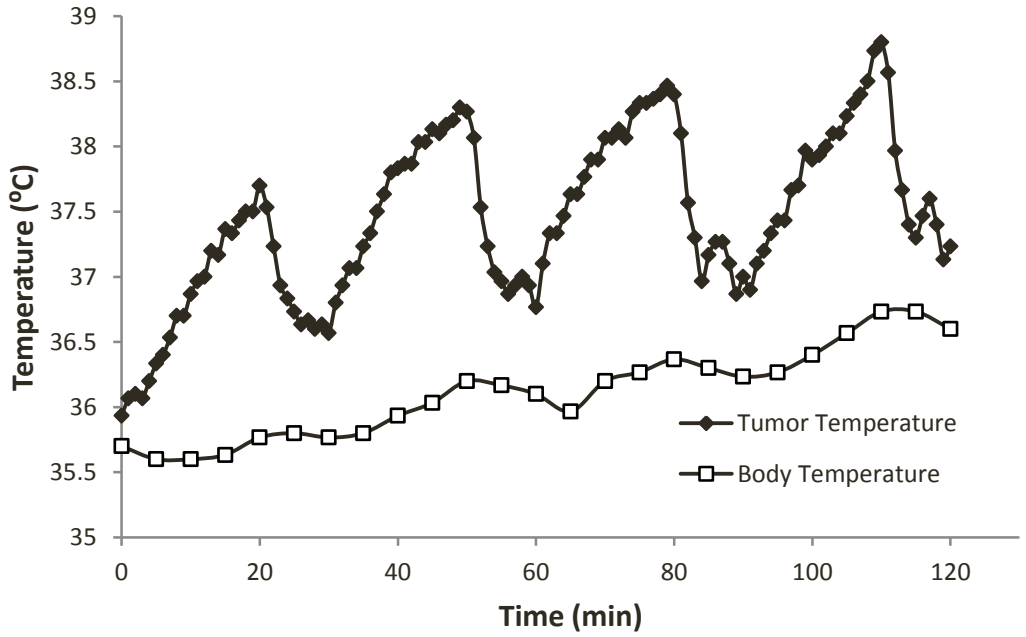
Inhibition of GBM Cell Proliferation

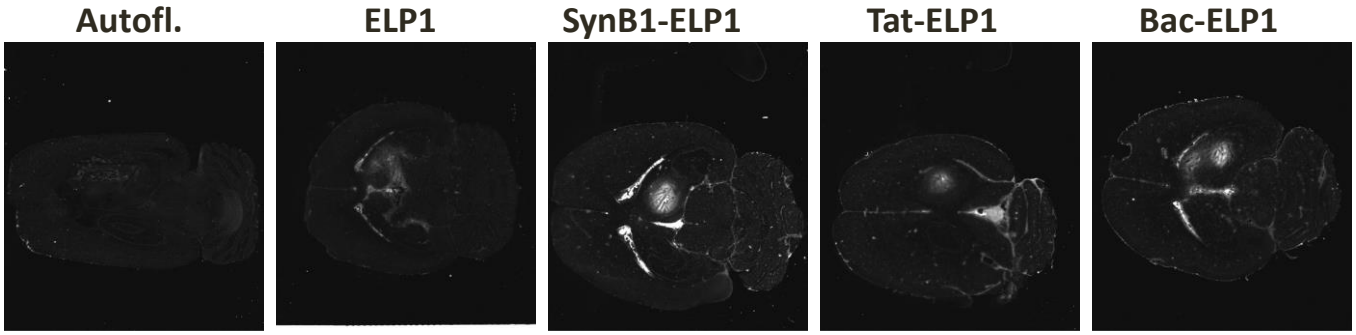


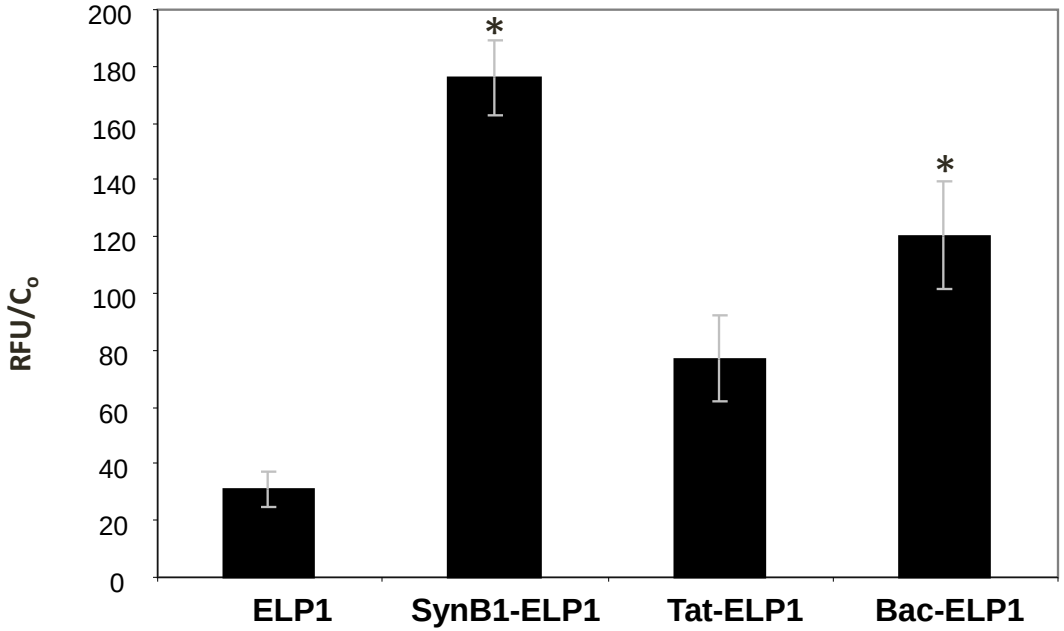


Heating Intracerebral GBM Tumors

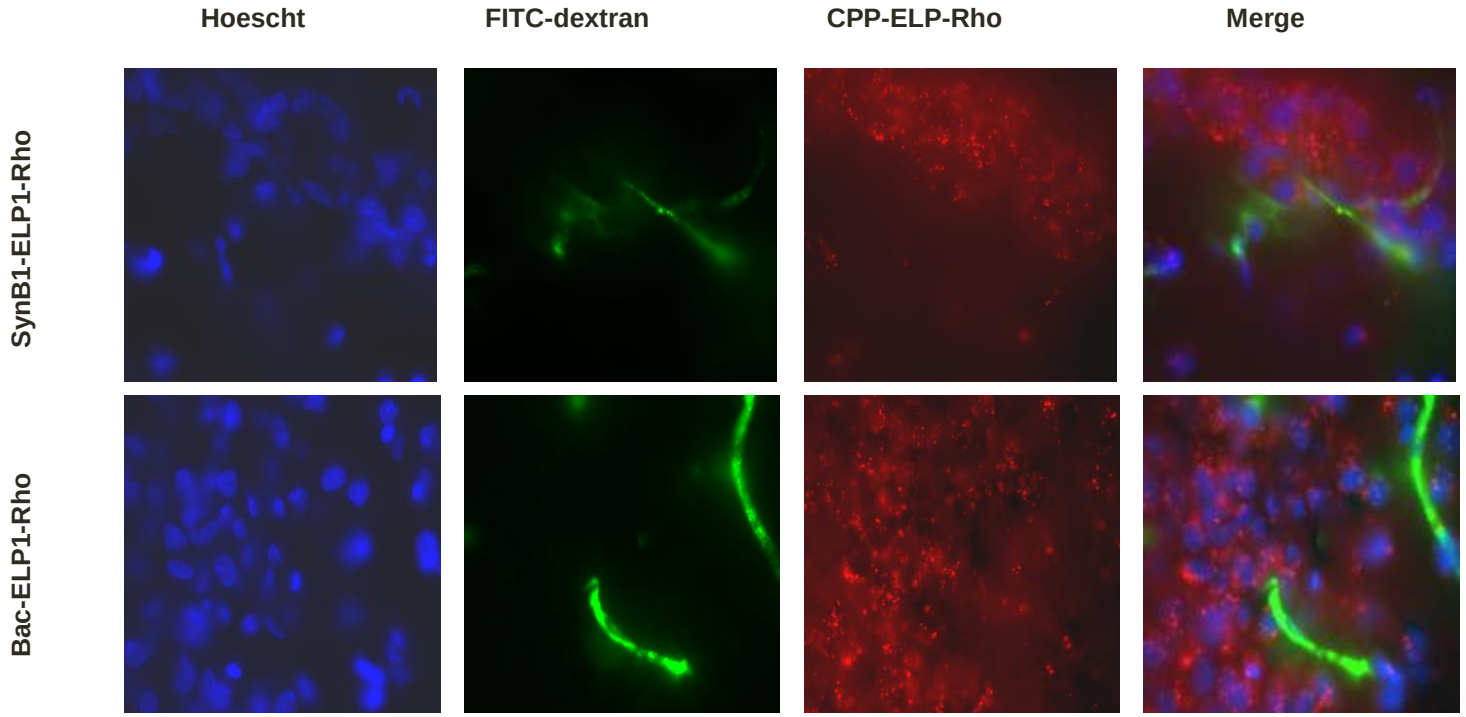


Delivery to Intracranial C6 Tumors

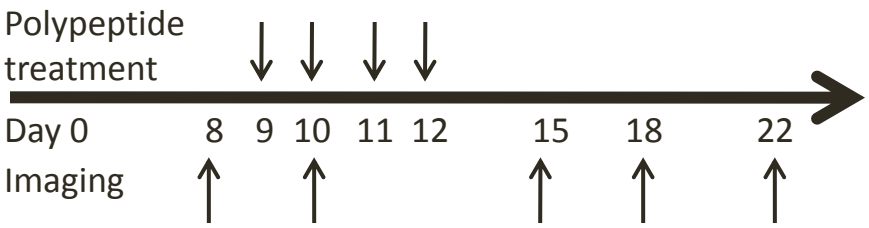


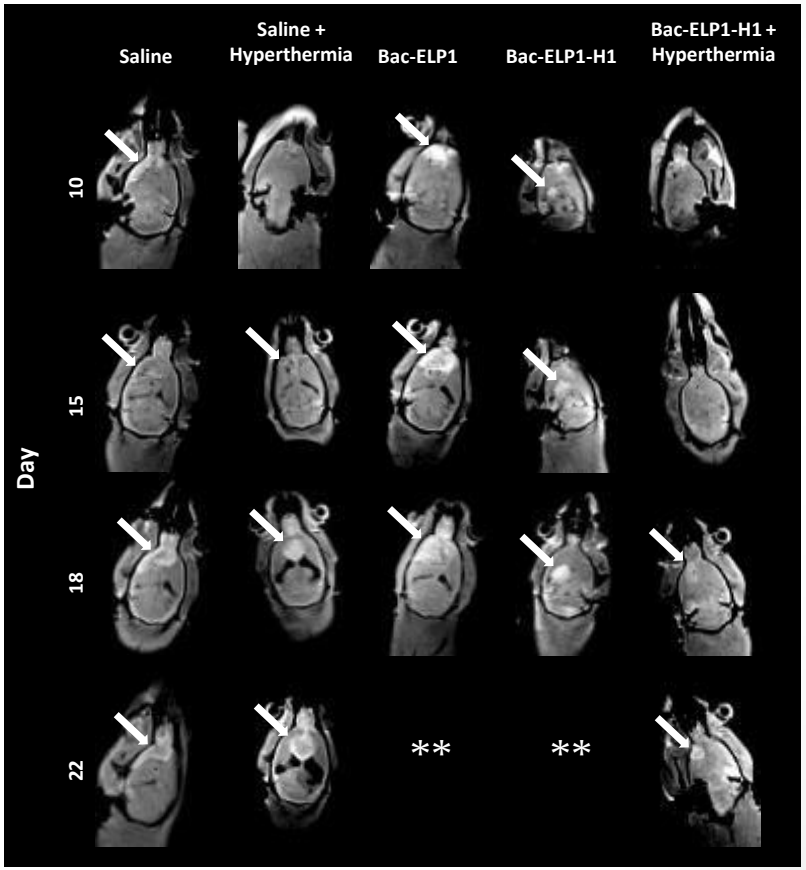


Delivery to Intracranial C6 Tumors



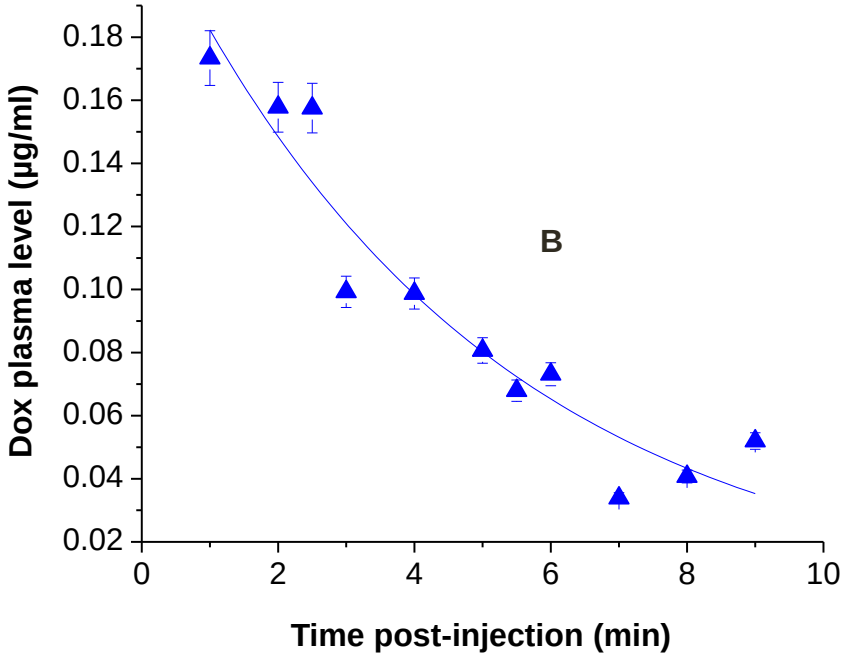
Inhibition of Intracranial C6 Tumor Growth



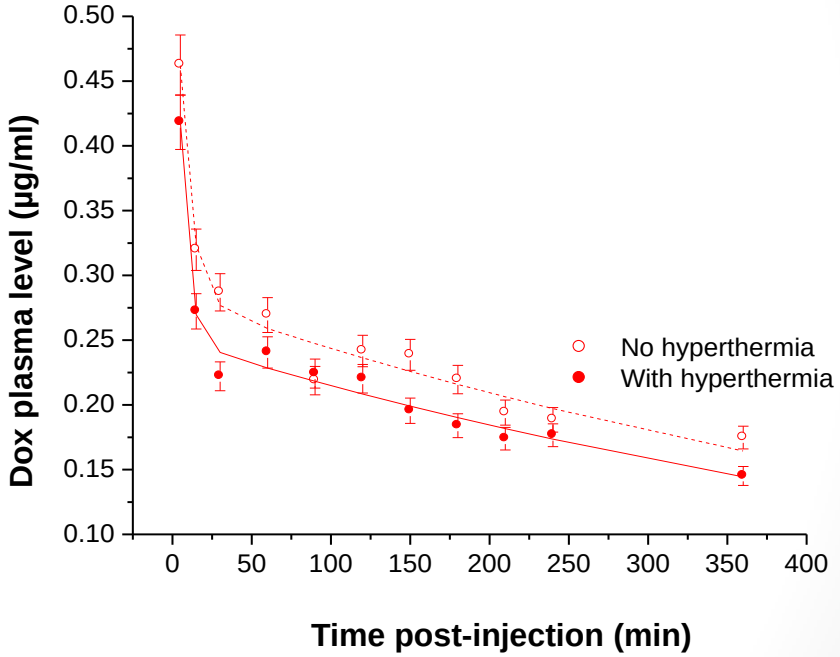


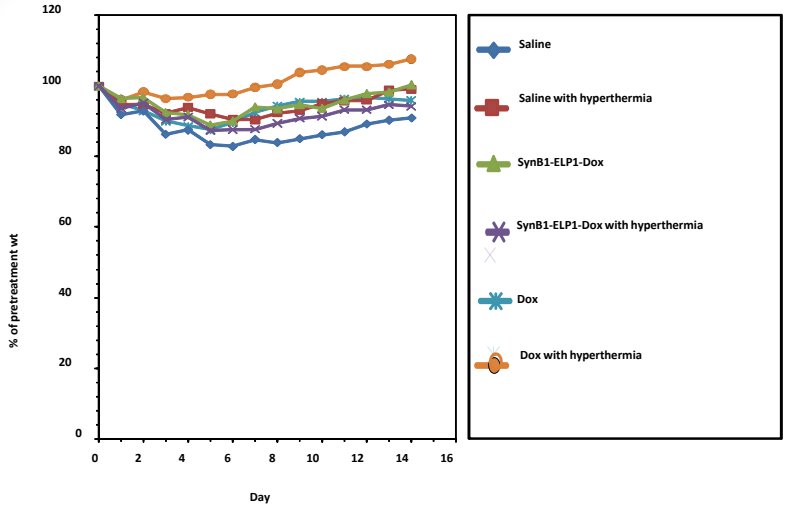
Plasma Clearance

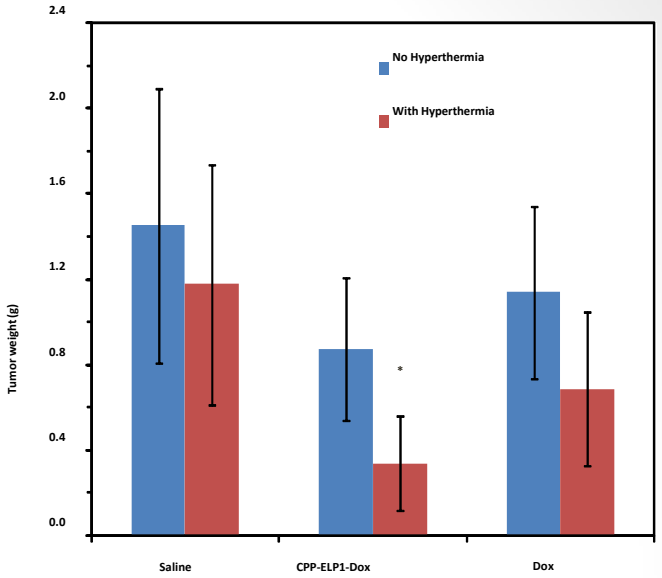
Free Dox

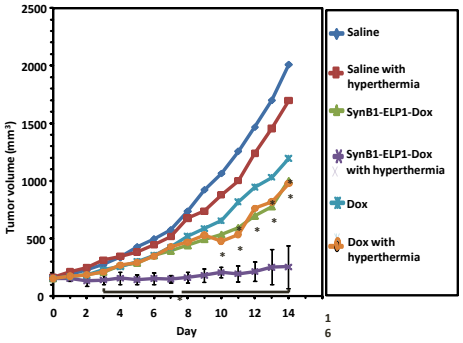


Delivery of Dox with ELP




120
100
80
60
40
20
0
% of pretreatment wt
0
2
4
6
8
10
12
14
16
Day
Saline
Saline with hyperthermia
SynB1-ELP1-Dox
SynB1-ELP1-Dox with hyperthermia
Dox
Dox with hyperthermia


2.4
2.0
1.6
1.2
0.8
0.4
0.0
Tumor weight (g)
No Hyperthermia
With Hyperthermia
*
Saline
CPP-ELP1-Dox
Dox


2500
2000
1500
1000
500
0
Tumor volume (mm³)
0
2
4
6
8
10
12
14
16
Day
Saline
Saline with hyperthermia
SynB1-ELP1-Dox
SynB1-ELP1-Dox with hyperthermia
Dox
Dox with hyperthermia