STIMULI-SENSITIVE COMBINATION NANOPREPARATIONS OF siRNA AND CHEMOTHERAPEUTIC DRUGS TO TREAT MULTIDRUG RESISTANT CANCER

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The idea of combination therapy of multidrug resistant (MDR) tumors is based on using siRNA down-regulating proteins involved in tumor defense mechanisms (Pgp, survivin, Bcl2) together with traditional chemotherapeutics.

Problems: - stabilization and delivery siRNA in vivo;

- synchronization of drug and siRNA;
- co-loading of a drug and siRNA on the same nanocarrier

Challenges with siRNA delivery

Even after almost 15 years since RNAi was described by Fire and Mello, there are no FDA approved, siRNA-based therapies, for the treatment of cancer. Eight siRNA-based formulations, for cancer therapy, are currently in the different phases of clinical trials*

Problems**

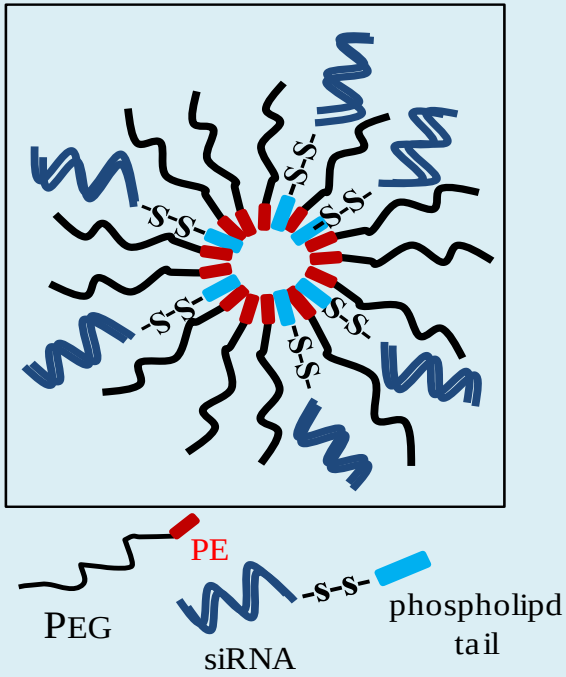
✓Rapid degradation by serum nucleases

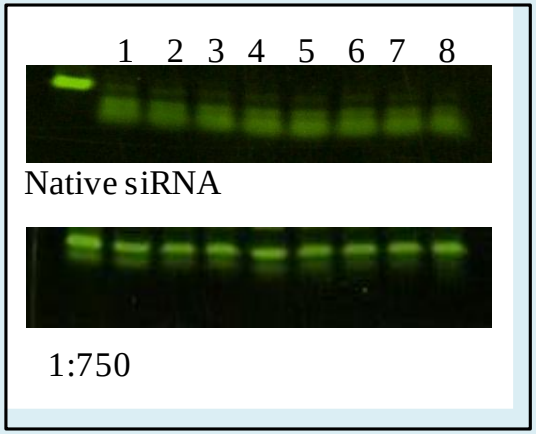
✓Poor cellular uptake due to inherent anionic charge

* Davidson, B. L., & McCray, P. B. (2011). Current prospects for RNA interference-based therapies. Nature Reviews Genetics, 12(5), 329-340.

** Adapted from Navarro, G., S. Essex et al. Drug Delivery and translational medicine (2011)

New system for effective stabilization and delivery of siRNA: Reversible siRNA-phospholipid conjugate in PEG-PE polymeric mixed micelles

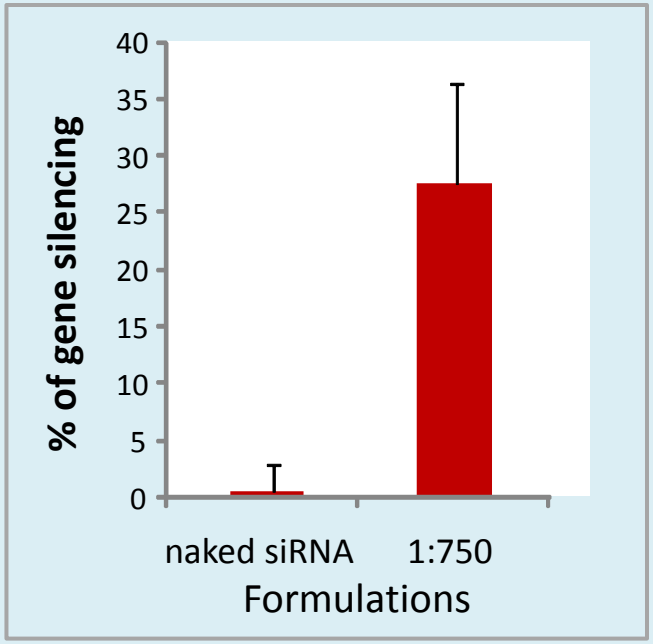




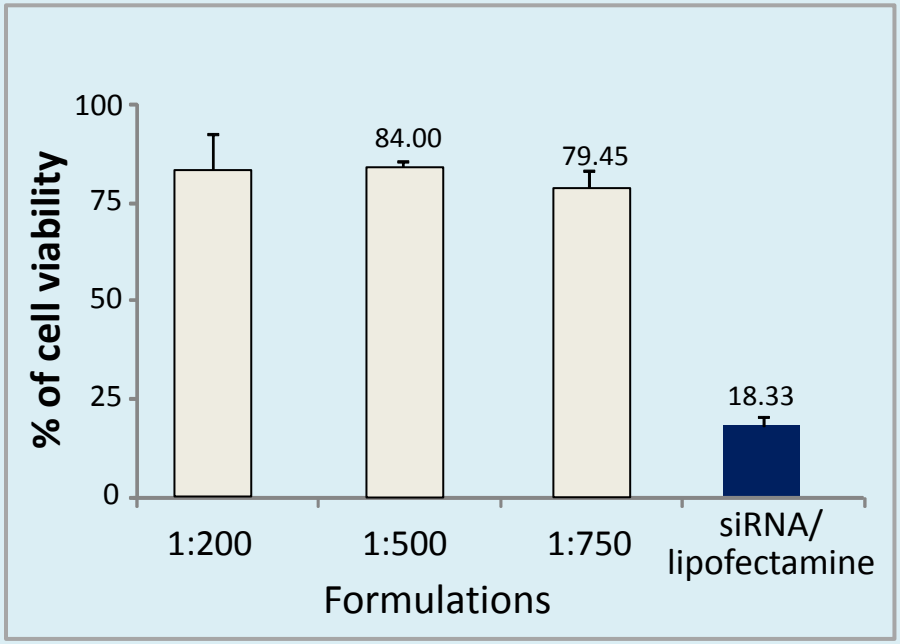
<u>Left panel</u>: Schematic structure of siRNA-PE/PEG-PE mixed micelles.

<u>Right panel</u>: stability of siRNA against nucleolysis in 1:750 mixed micelles compared to that of the free siRNA at different time-points till 24 h

Gene silencing of different formulations containing a 84 nM concentration of siRNA

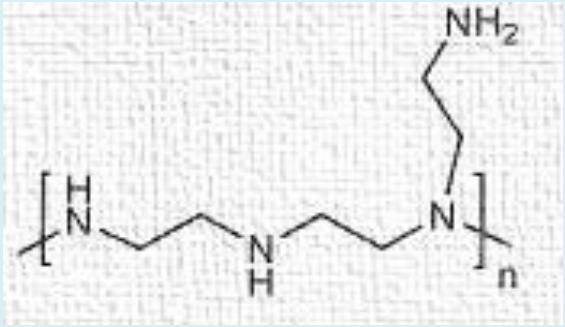


Cell viability on GFP-C166 after a 48 h incubation of different siRNA formulations



Left panel: % of gene silencing induced in GFP-C166 cells (comparison between naked siRNA and siRNA-PE in 1:750 mixed micelles formulation).
Right panel: cell viability in the presence of various siRNA-PE-containing PEG-PE-based formulations in comparison with same amount of siRNA used as the Lipofectamine formulation.

Polyethylenimine (PEI)-based siRNA micelle-like nanocarriers



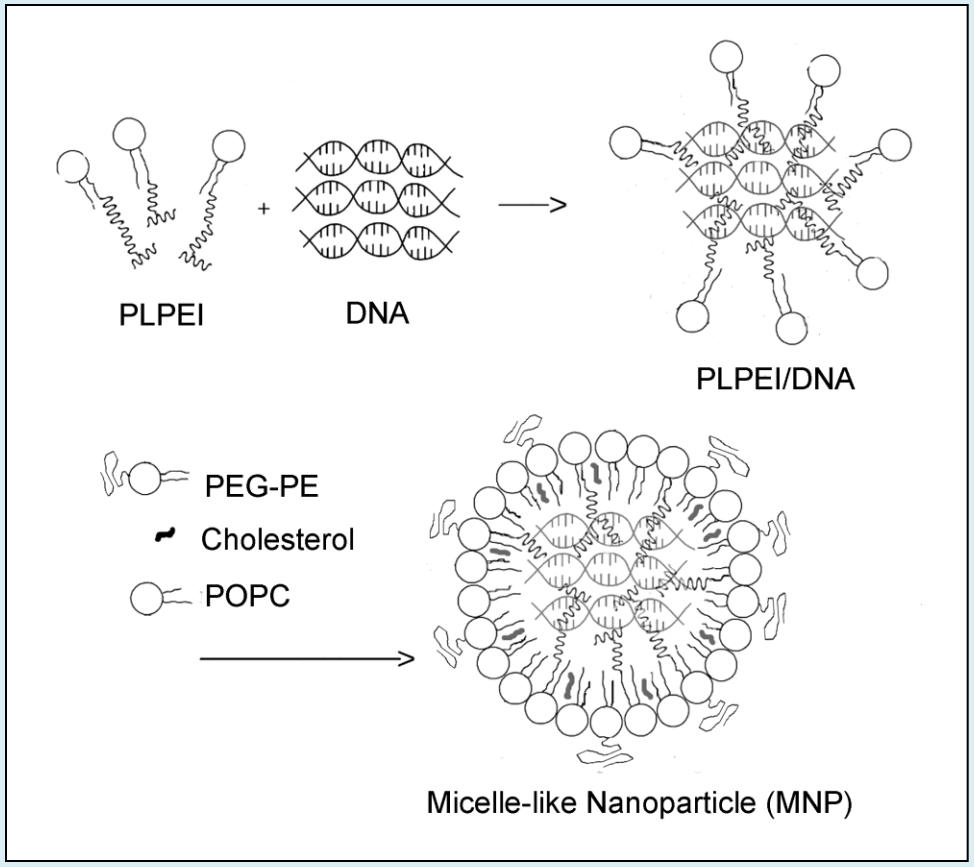
Pros*

- Proton sponge effect due to cationic nature
- Synthetic flexibility (Linear/branched)
- Cationic charge condenses siRNA and facilitates cell uptake
- Low molecular weight PEI (1.8 kDa) is non-toxic

Cons*

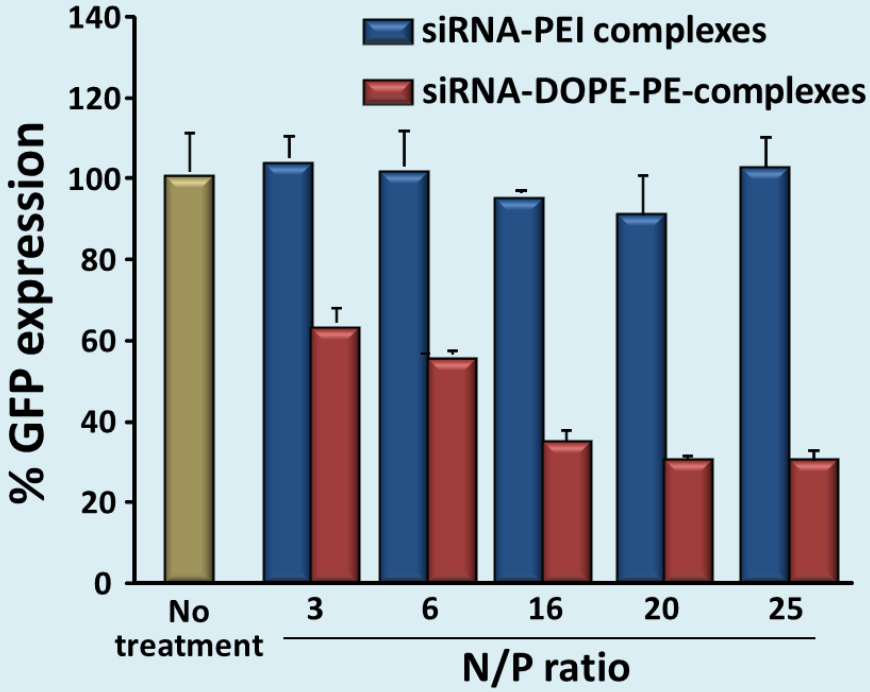
- Toxicity (High molecular weight > 25kDa)
- Non-specific interaction with serum proteins
- RES mediated removal

Self-assembly PEI-lipid nanoparticles



- Driven by electrostatic interaction (DNA/siRNA complexation) followed by hydrophobic interaction (formation of lipid monolayer coat)
- Simple and quantitative DNA/siRNA loading procedure
- High DNA/siRNA loading capacity (30 wt%)
- Combine polyplexes with sterically-stabilized micelles

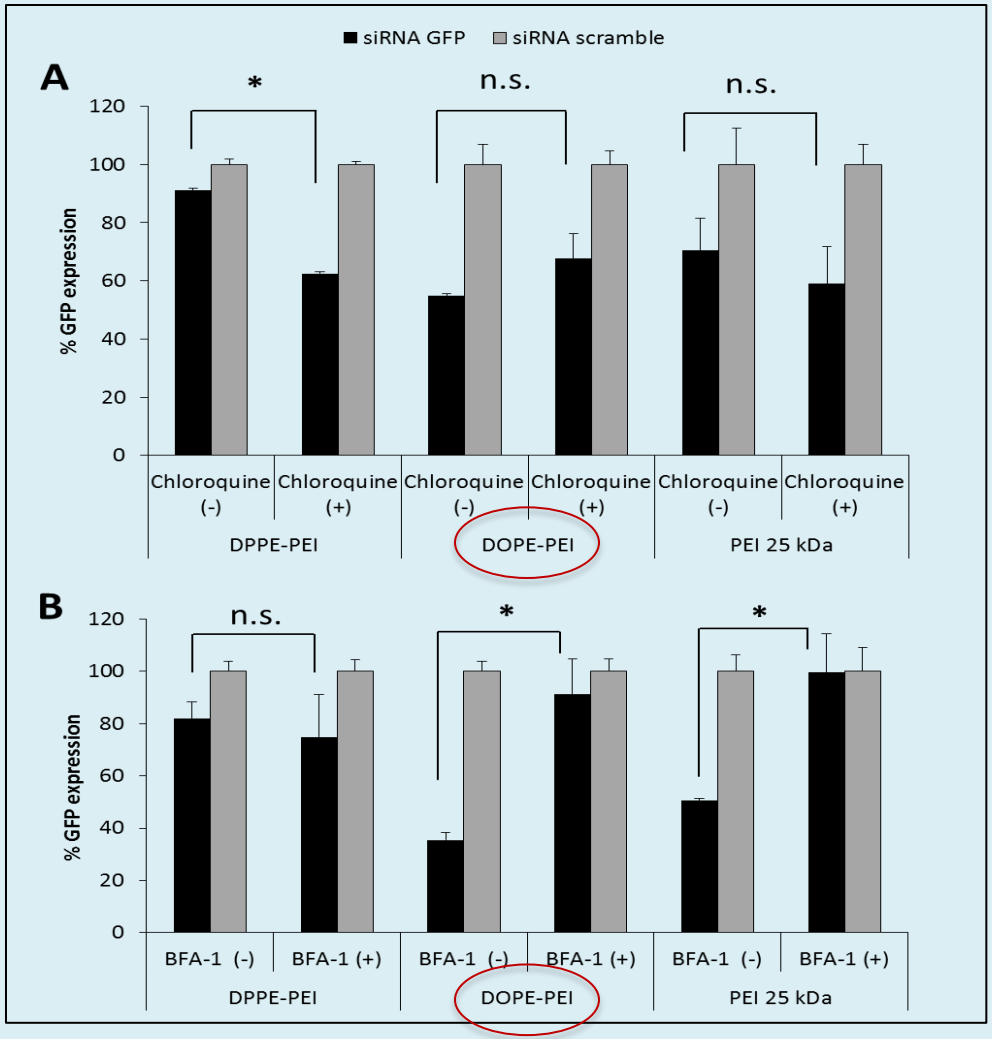
Gene silencing efficacy of the PEI–lipid/siRNA(GFP) complexes in cells that stably express GFP



GFP flourescence was measured by cytometry. The absence of GFP suppression was observed for non-modified PEI complexes, while a 75 % GFP signal reduction was seen for PEI-PE complexes.

Endosomal escape of DOPE-PEI

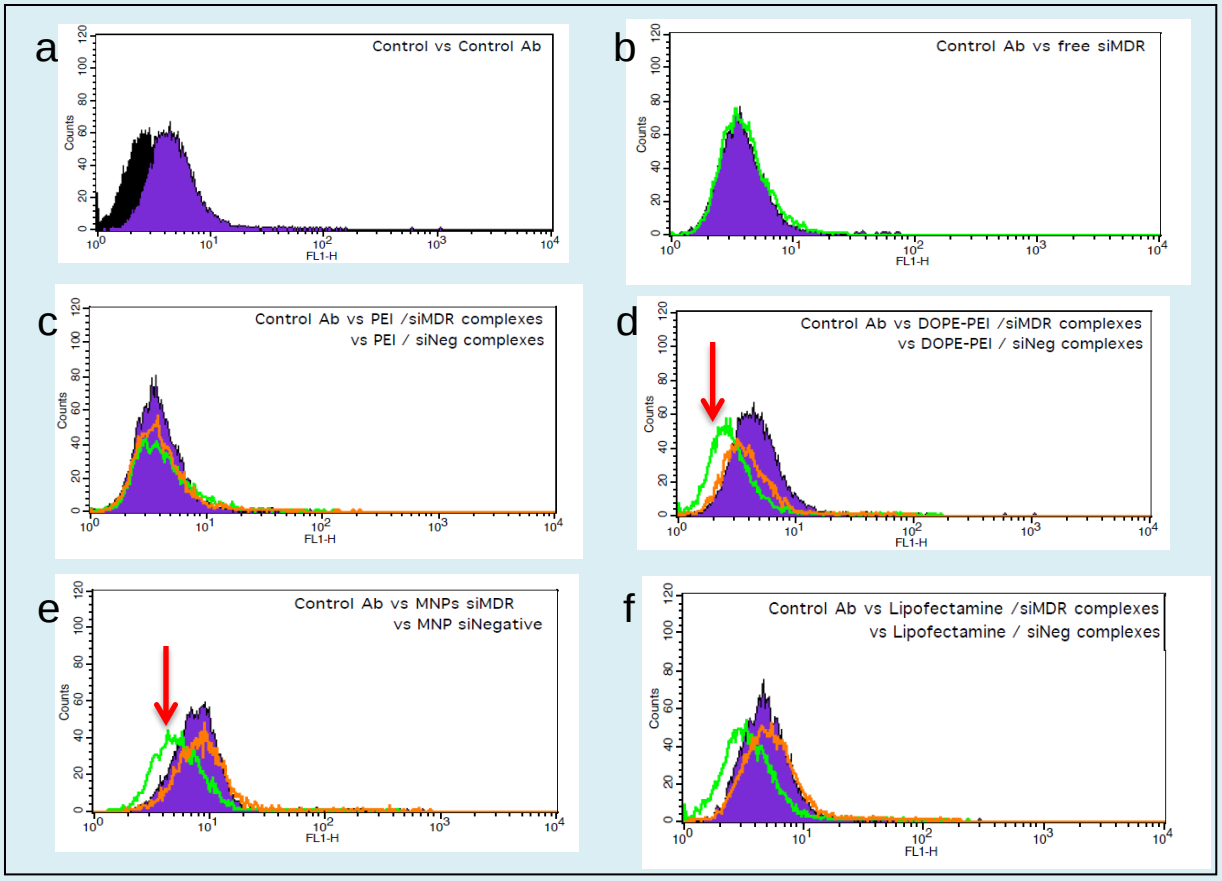
- ✓ Positive chloroquine lysomotropic dependency seen in case of DPPE-PEI not in the case of DOPE-PEI
- ✓ Bafilomycin abolished the GFP downregulation ability of DOPE-PEI and not DPPE-PEI
- ✓ The greater efficacy of DOPE-PEI in the gene downregulation is due to its ability for endosomal escape



Navarro, Essex et al. , Nanomedicine, (USA) 2013

P-glycoprotein (P-gp) downregulation by DOPE-PEI-based nanocarriers

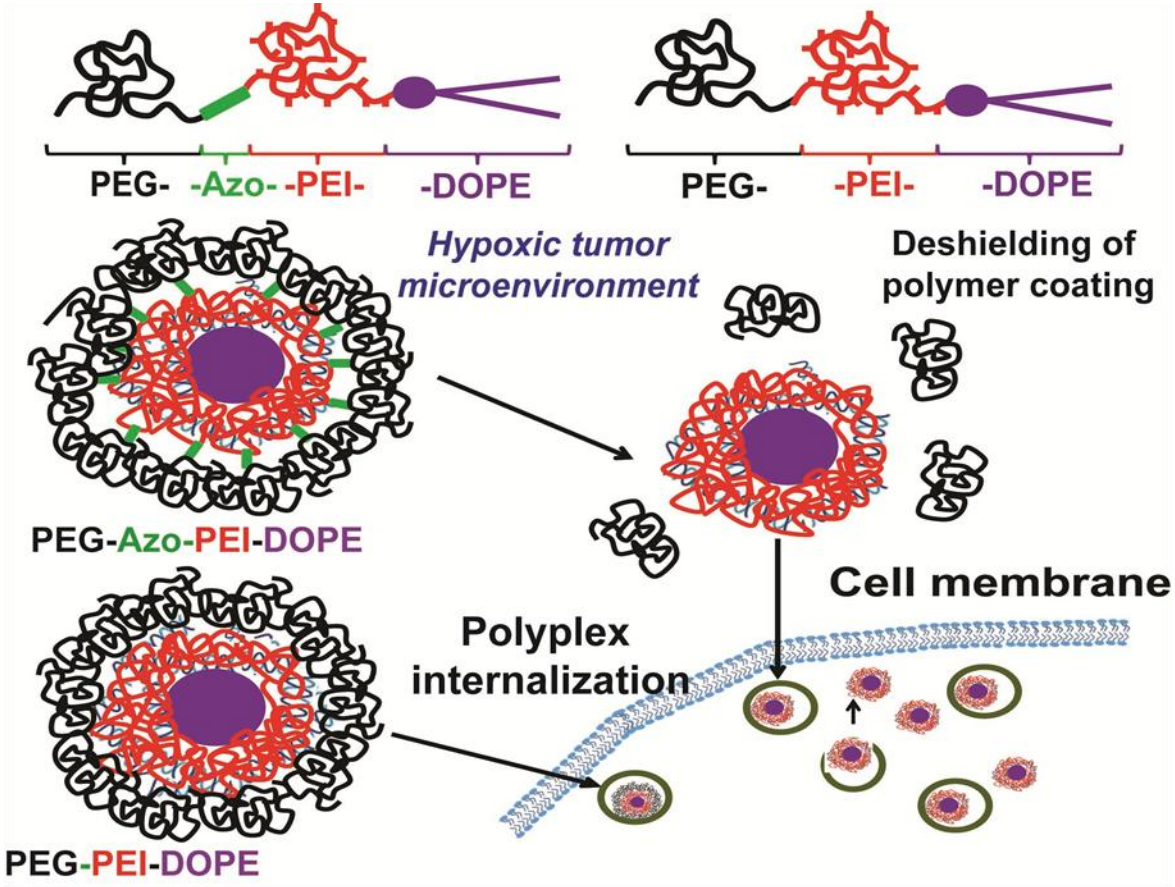
- ✓ The *in vitro* tumor model chosen was the drug resistant, human breast cancer MCF7/ADR cells
- ✓ DOPE-PEI-based complexes and MNPs containing siMDR1 (siRNA targeting MDR1 gene) successfully downregulated the surface expression of P-gp



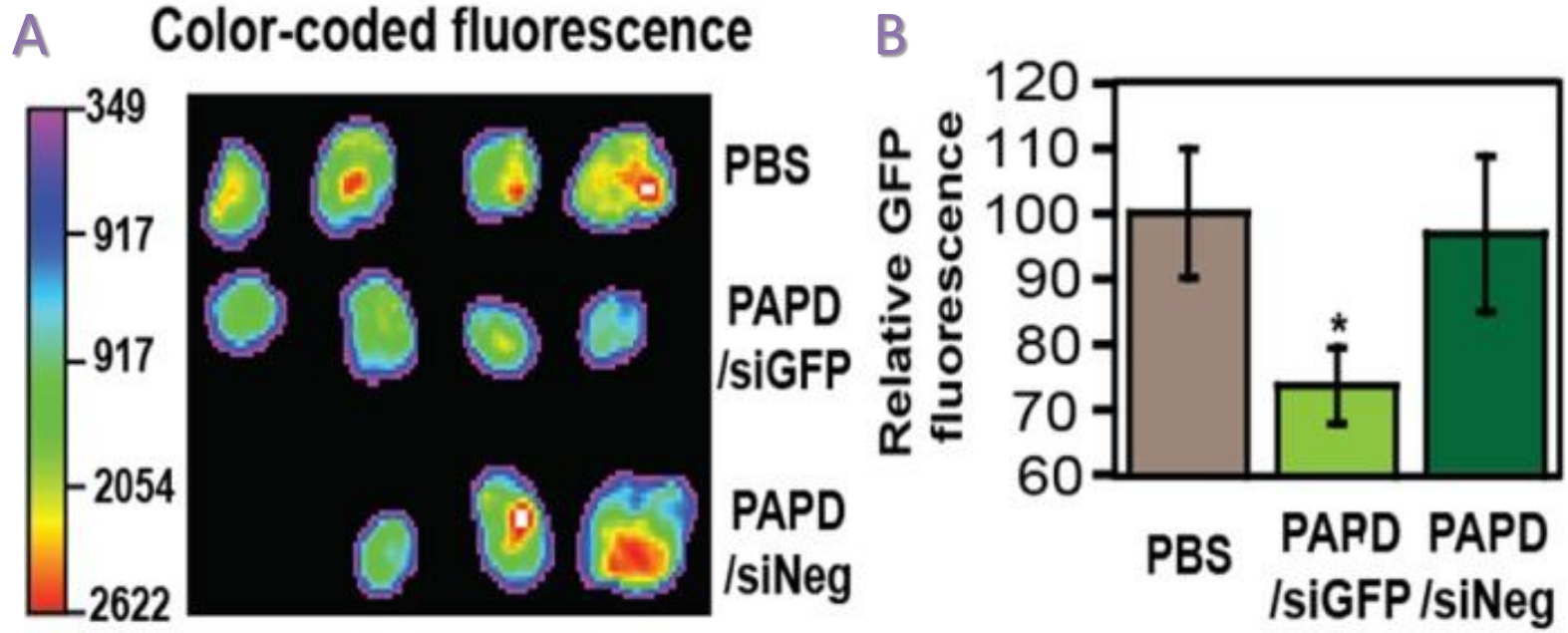
Navarro, Essex et al., Nanomedicine (Lond.), 2012

ADDING STIMULI SENSITIVITY

A schematic structure of the nanosystem to target siRNA and drugs to hypoxic areas in tumors



In vivo silencing activity



A - *Ex vivo* fluorescence optical imaging of tumors 48h after injection of PBS , PEG-Az-PEI-PE/anti-GFP siRNA complexes (PAPD/siGFP, n=4), PEG-Az-PEI-DOPE/negative siRNA complexes

B - Cell-associated fluorescence of dissociated tumors by flow cytometry

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ARTICLE

Matrix Metalloprotease 2-Responsive Multifunctional Liposomal Nanocarrier for Enhanced Tumor Targeting

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PNAS

Enhanced anticancer activity of nanopreparation containing an MMP2-sensitive PEG-drug conjugate and cell-penetrating moiety

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Edited by Alexander M. Klibanov, Massachusetts Institute of Technology, Cambridge, MA, and approved September 3, 2013 (received for r March 15, 2013)

In response to the challenges of cancer chemotherapeutics, including poor physicochemical properties, low tumor targeting, insufficient tumor cell internalization/bioavailability, and side effects, we developed a unique tumor-targeted micellar drug-delivery platform. Using water solubility, off-target toxicity, and acquired dr Among many attempts to deal with these issues polymeric micelles have led to successes in deliver However, the low drug loading (10), risk of prematur

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Matrix metalloproteinase 2-sensitive multifunctional polymeric micelles for tumor-specific co-delivery of siRNA and hydrophobic drugs

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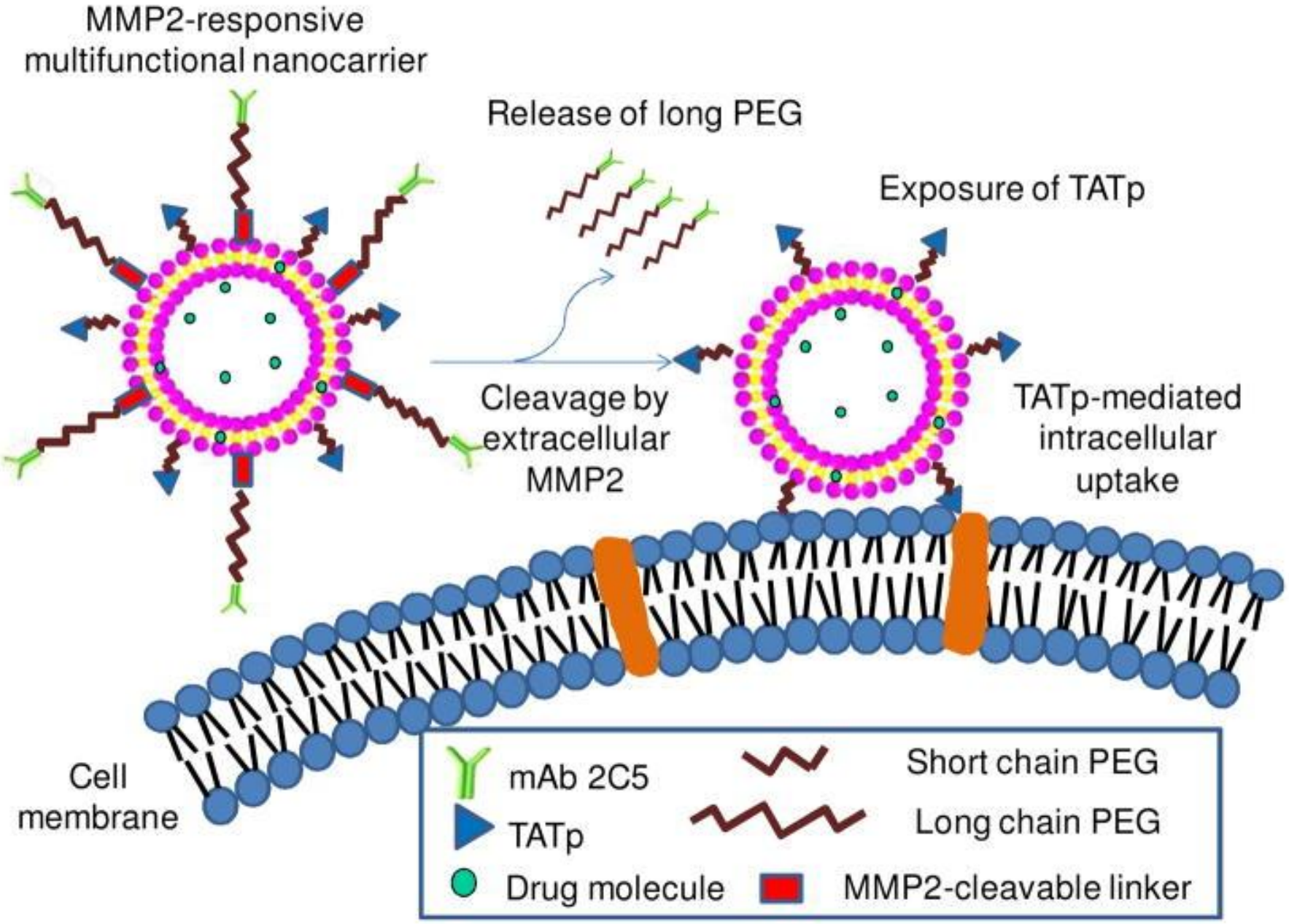
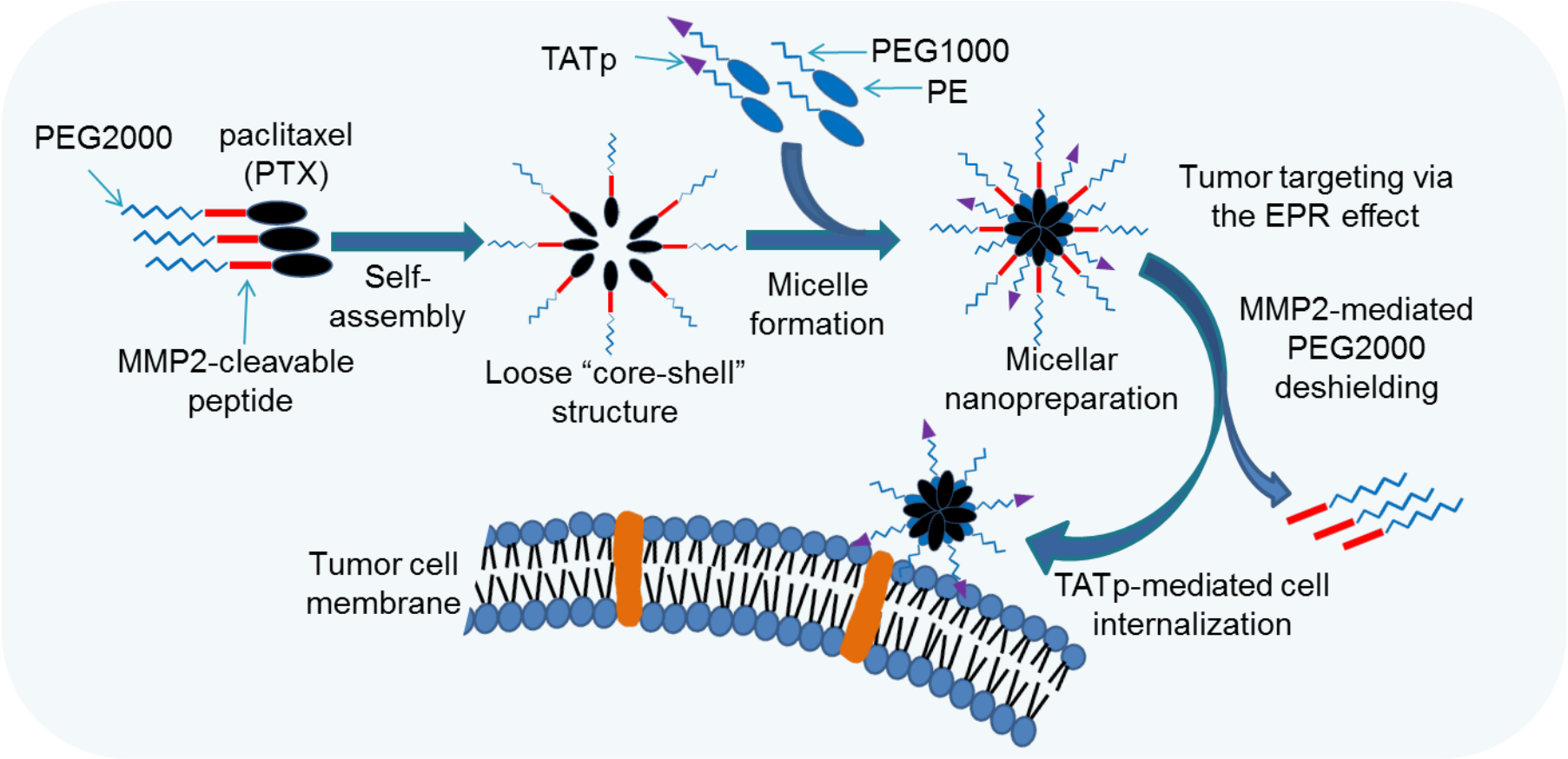

MMP2-responsive
multifunctional nanocarrier
Release of long PEG
Exposure of TATp
Cleavage by
extracellular
MMP2
TATp-mediated
intracellular
uptake
Cell
membrane
mAb 2C5
TATp
Drug molecule
Short chain PEG
Long chain PEG
MMP2-cleavable linker

Nanopreparation Containing MMP2-sensitive PEG-paclitaxel Conjugate and Cell Penetrating Moiety



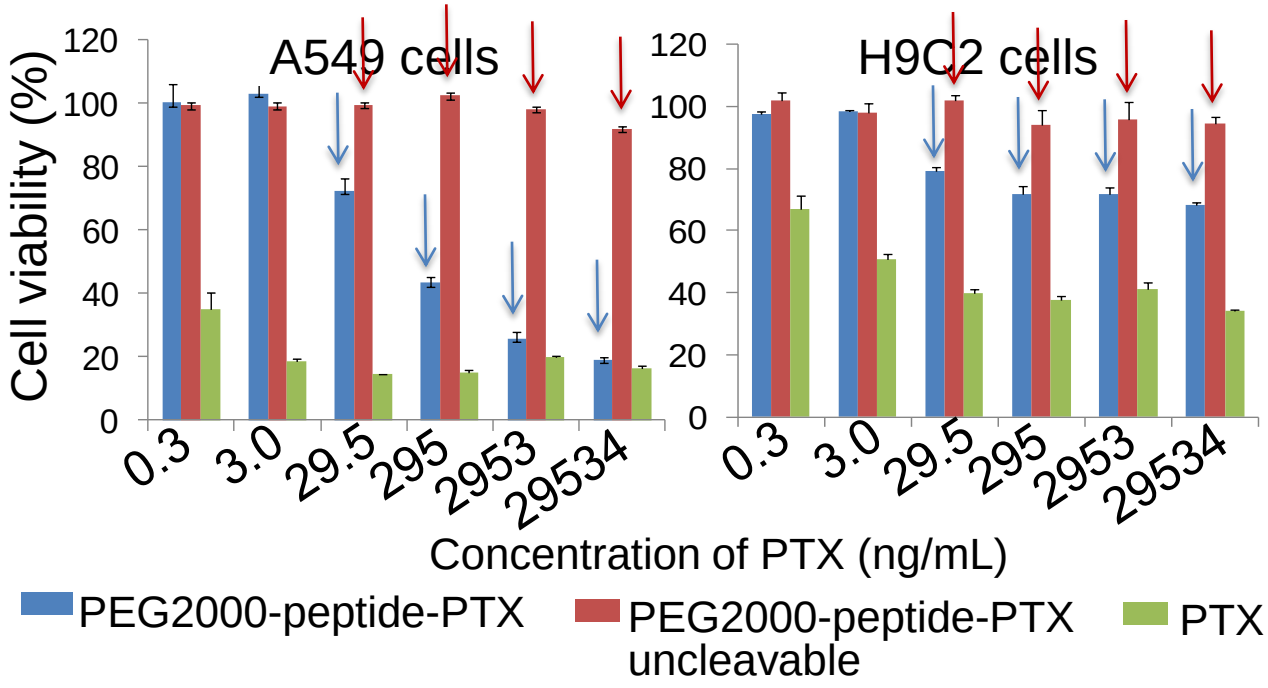
PE, phosphatidylethanolamine

PEG1000-PE: a building block for nanocarrier
TATp-PEG1000-PE: a cell-penetrating moiety
PEG2000-peptide-PTX: (1) an MMP2-cleavable prodrug
(2) a self-assembly building block

Zhu L, et al. (2013), PNAS, 110(42):17047-52.
Zhu L, et al., Patent No. PCT/US2013/072216.

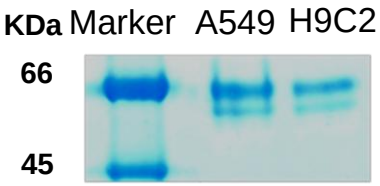
MMP2-triggered Tumor Cell-specific Cytotoxicity

A



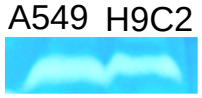
B

SDS-PAGE



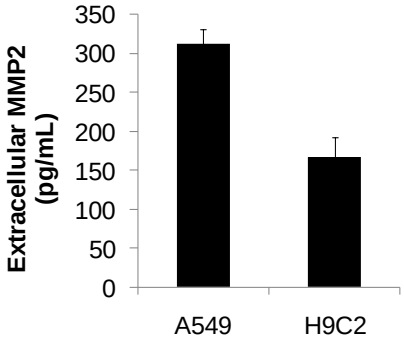
C

Zymography



D

MMP2 ELISA



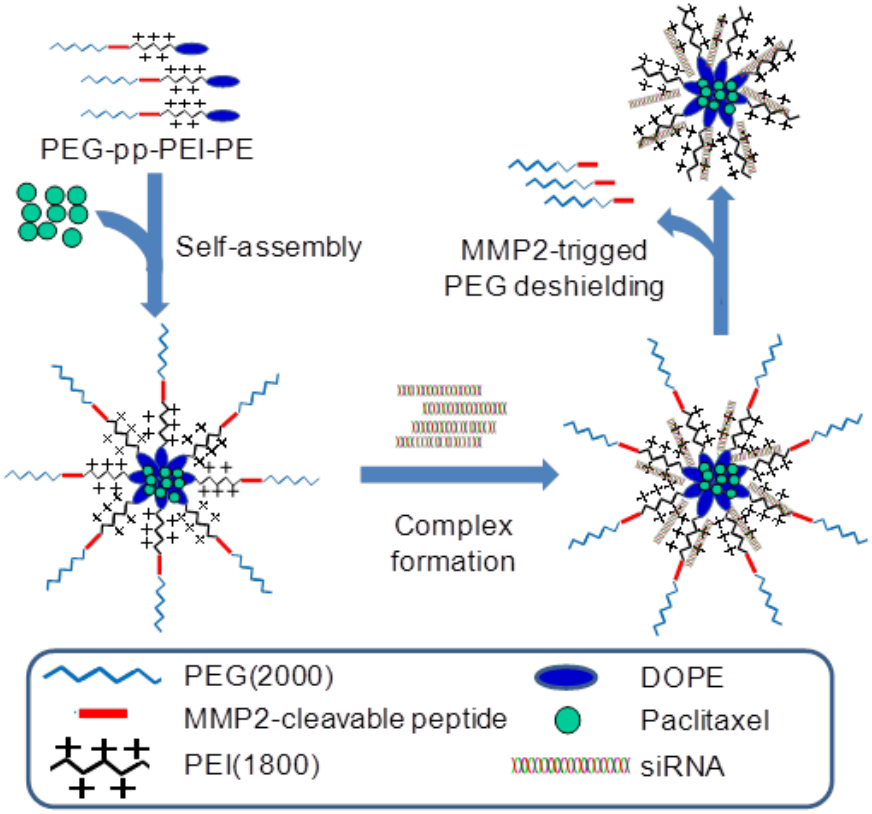
Cytotoxicity in A549 and H9C2 cells (A)
Incubation time: 72h

MMP2 secretion
The 2-day media was concentrated and analyzed using SDS-PAGE (**B**) and zymography (**C**).
Human MMP2 (EMD Biosciences): 66.5K Da

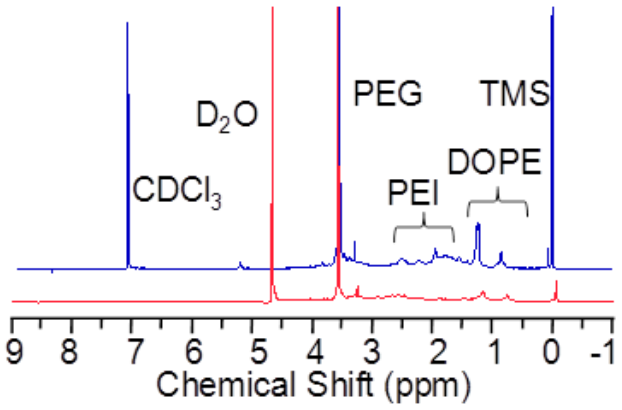
MMP2 ELISA (D)
The media was directly analyzed by MMP2 ELISA kit (sensitivity < 10pg/mL, Boster Immunoleader).

COMBINATION THERAPY

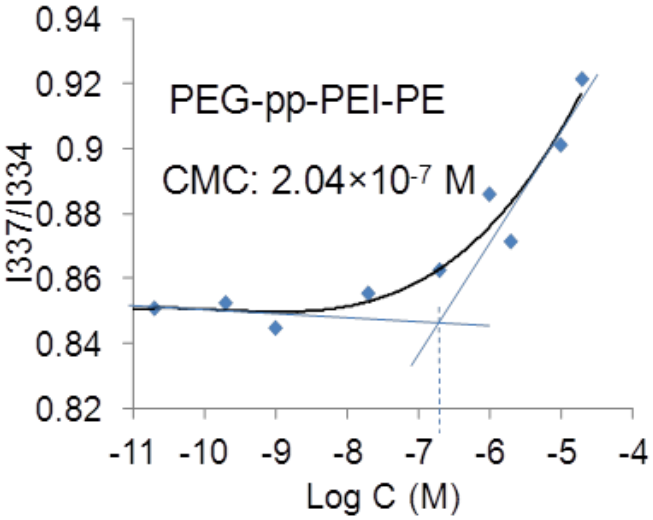
A



B



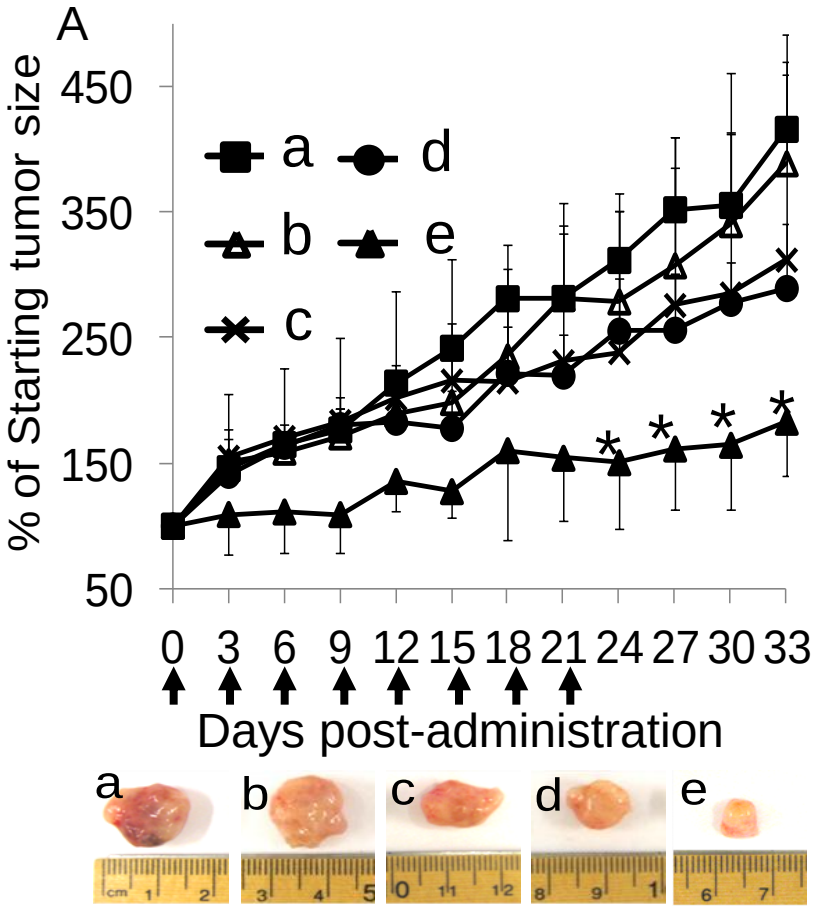
C

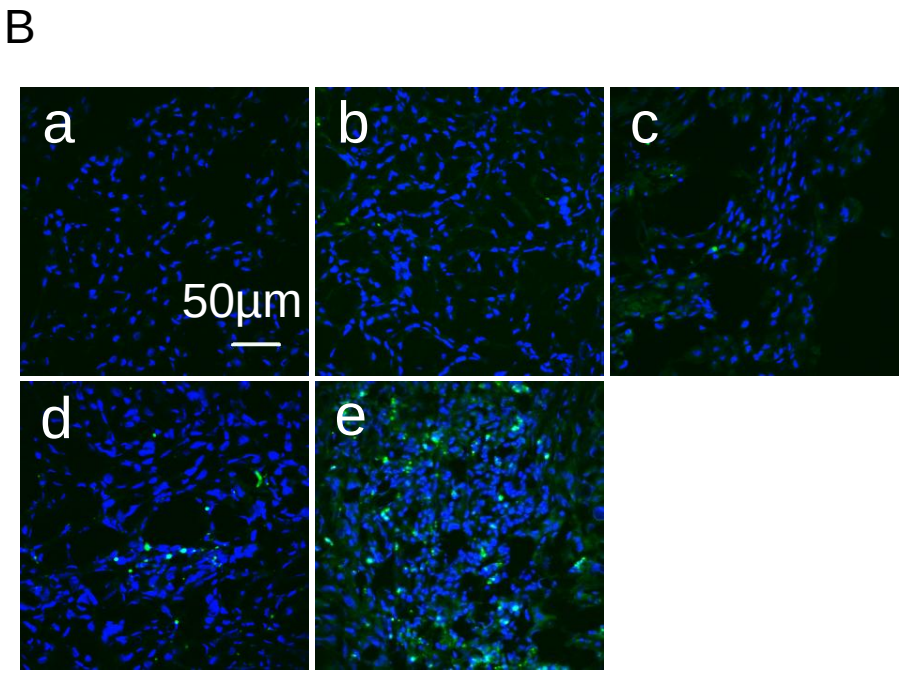


D

	pH	Particle size (nm)
PEG-pp-PEI-PE	5.5	16.7 ± 3.7
	7.4	16.5 ± 5.1
	9.0	21.7 ± 5.4

Tumor Growth Inhibition and Tumor Cell Apoptosis





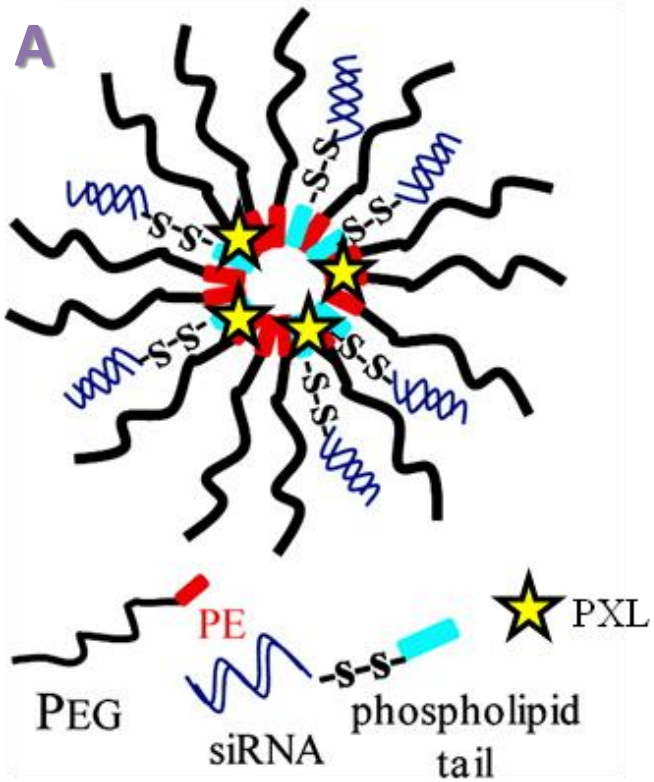
a, HBSS; b, TATp-PEG1000-PE/PEG2000-peptide-PTX (nonsensitive); c, PEG2000-PE/PTX; d, Free PTX; e, TATp-PEG1000-PE/PEG2000-peptide-PTX (MMP2-sensitive)

Tumor growth inhibition (A) and tumor cell apoptosis analyzed by TUNEL assay (B). Dose: 5mg/Kg PTX.

A . Schematic structure of survivin siRNA-S–S-PE/PXL PEG-PE mixed micelle.

B. Physic characteristics of survivin siRNA PM and PM containing survivin siRNA and PTX in combination (survivin siRNA/PXL PM)

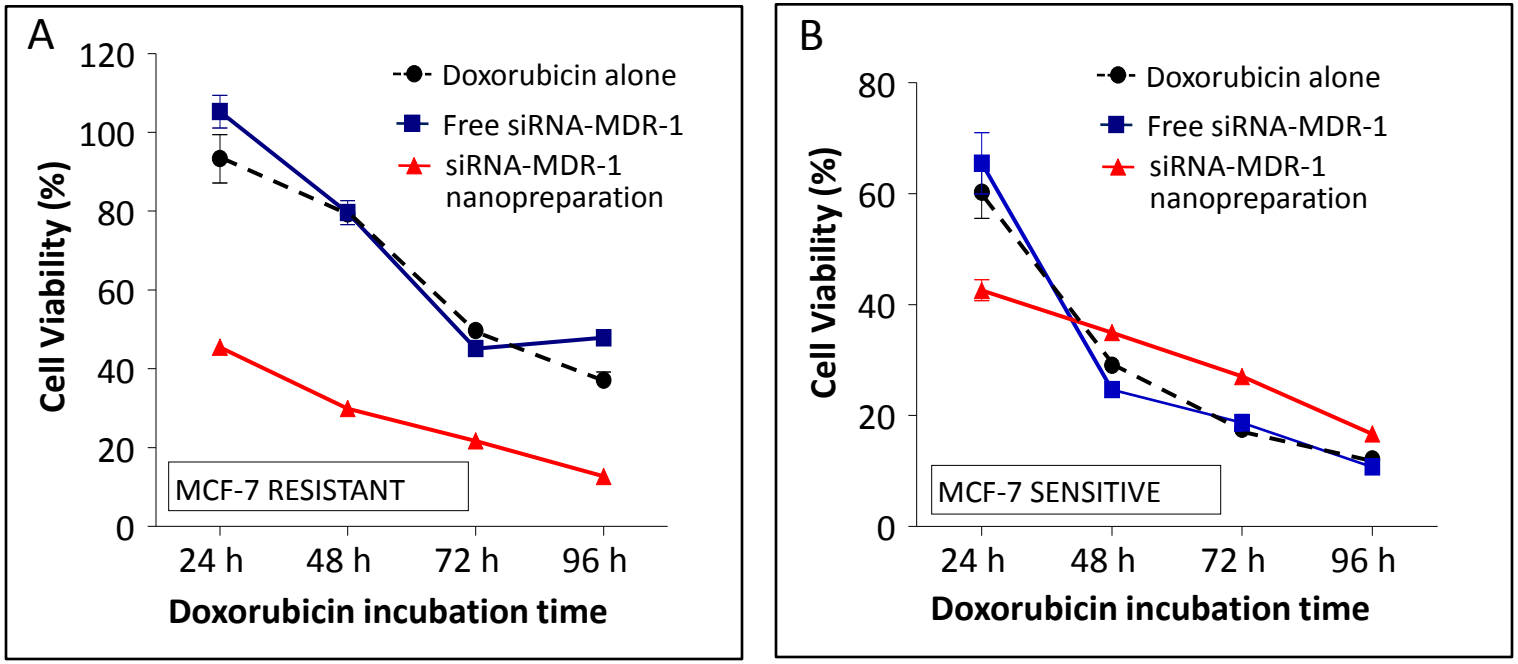
A



B

Formulation	Diameter (nm ± SD)	P.I. ± SD
Survivin siRNA PM	21.5 ± 3.3	0.160 ± 0.05
Survivin siRNA/PXL PM	25.0 ± 3.6	0.190 ± 0.07

Doxorubicin cytotoxicity in (A) resistant and (B) sensitive MCF-7 cells after treatment with siRNA nanopreparations



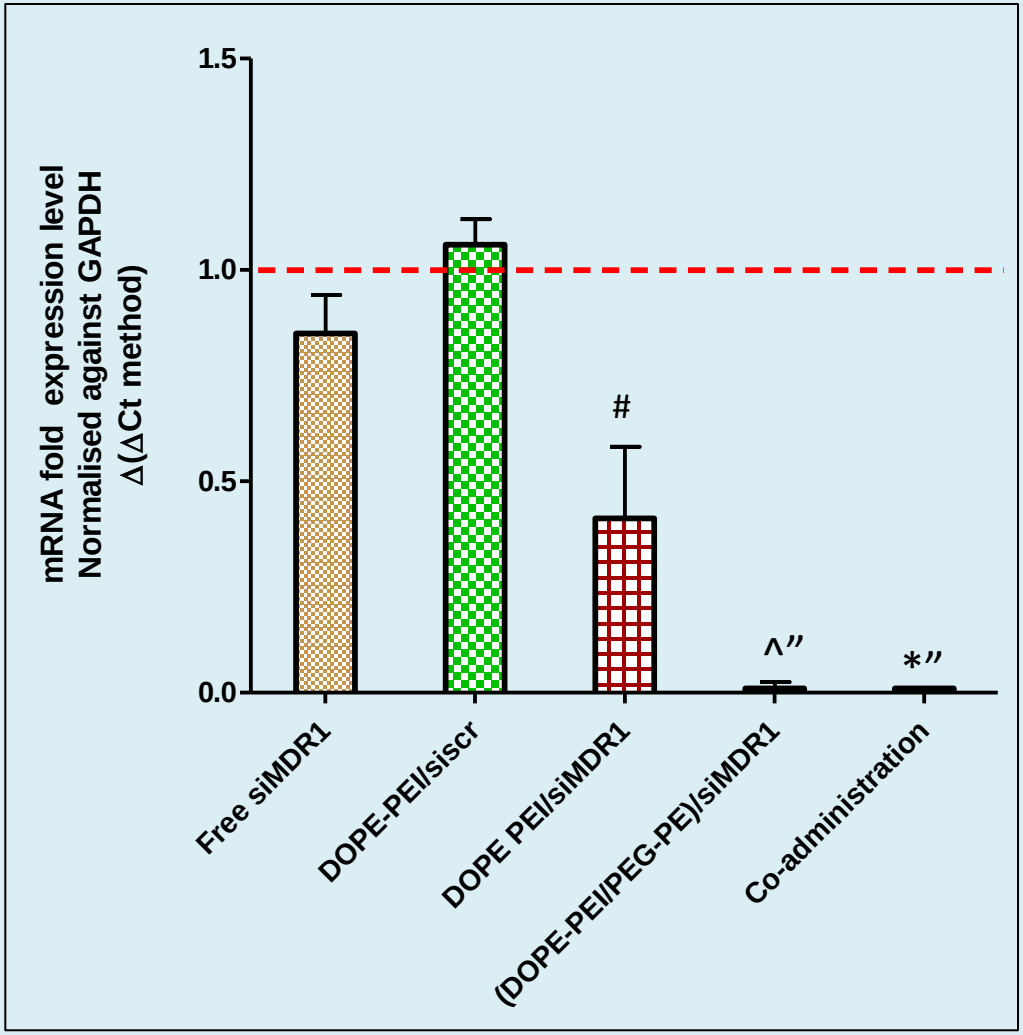
MCF-7 resistant and sensitive cells were treated with formulations prepared with siRNA targeting MDR-1 (siMDR). Cells were treated with doxorubicin (1µg/mL) for 24, 48, 72 and 96 h and cell viability was measured. Data are expressed as the mean ± SD (n=3).

PCR to evaluate MDR1 downregulation in tumors

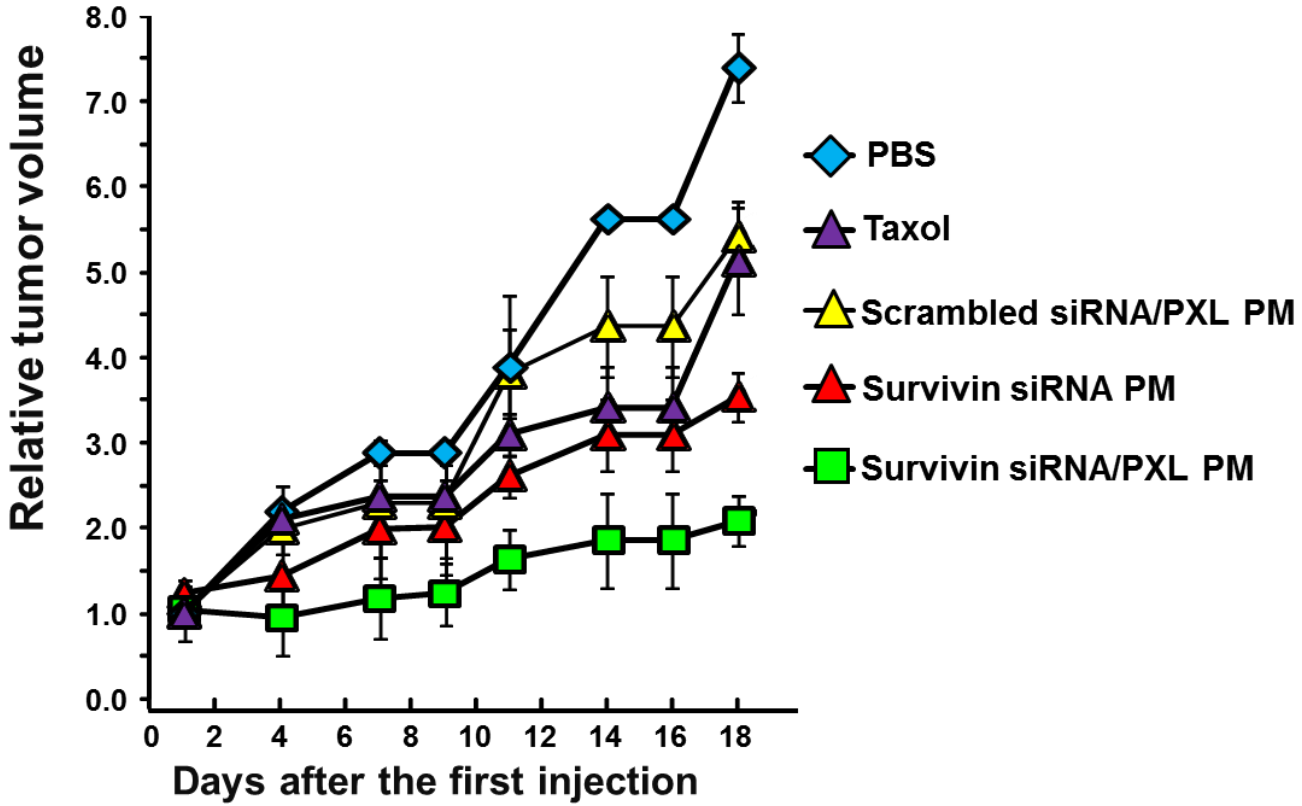
All treatment groups significantly down regulated the MDR1 gene with respect to the control groups

#, ^ and * - Respective treatment groups are statistically very significant than free siMDR1 and DOPE-PEI

” - Treatment groups statistically significant than DOPE-PEI/siMDR1

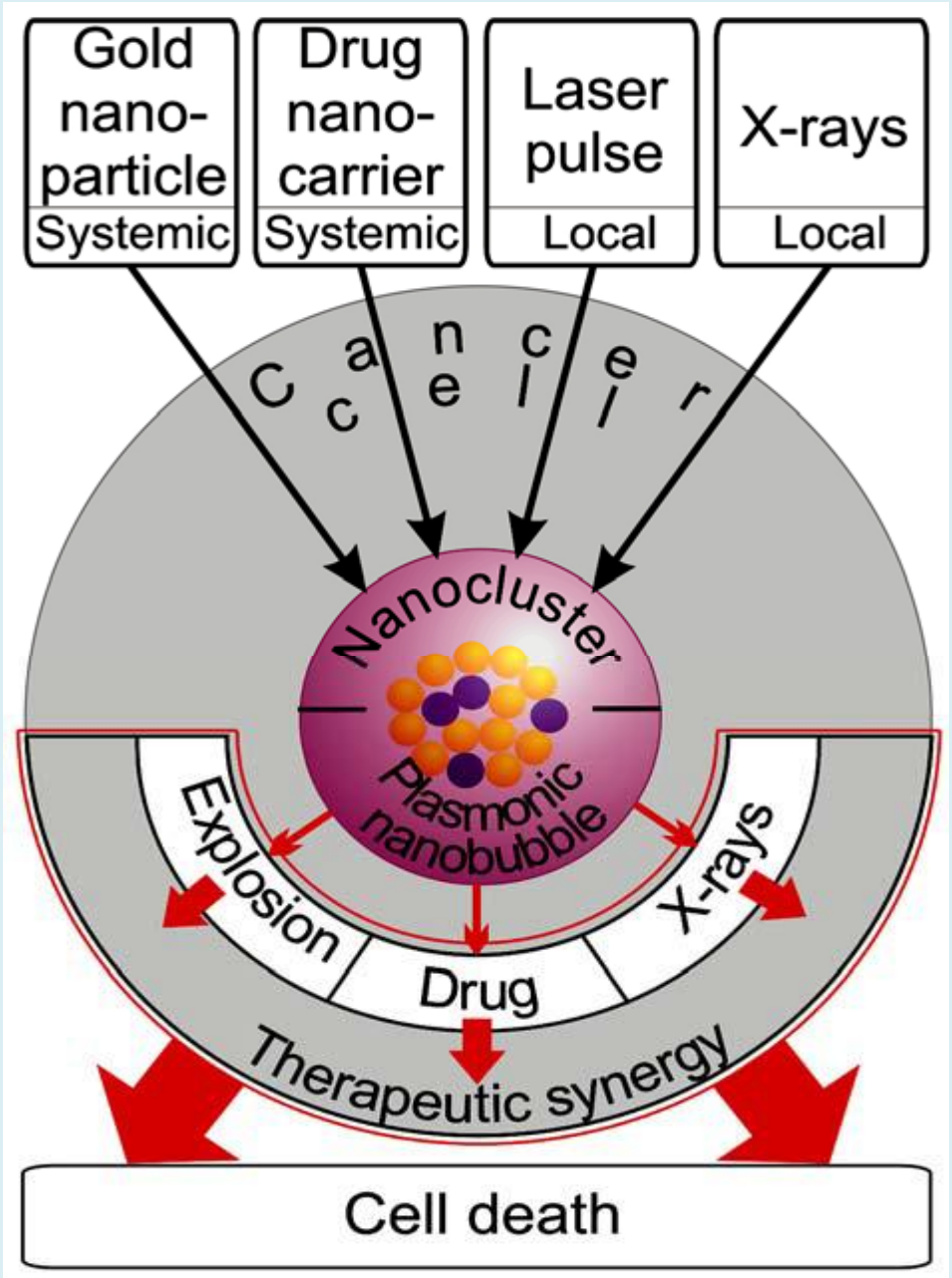


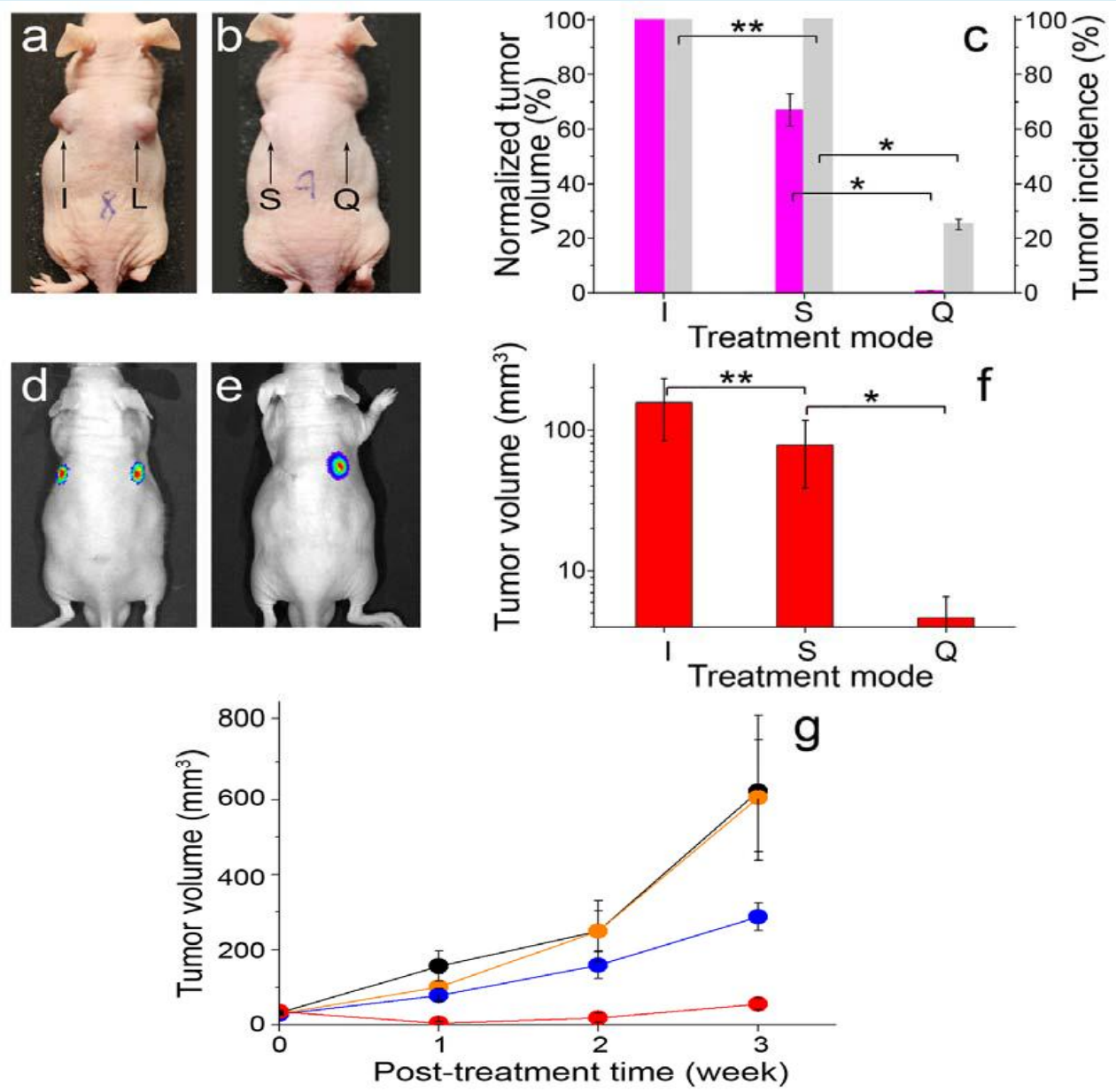
Therapeutic efficacy of NP containing survivin siRNA and PXL in combination on SKOV3-tr resistant ovarian cancer xenograft



**Relative tumor volume:* Tumor volume in mm^3 on day 'n' (Vn) / tumor volume at the start of the treatment (Vo) plotted versus time in days.

NEW CONCEPTS IN COMBINATION THERAPY USING LIPOSOMAL DRUGS


Gold nano-particle
Systemic
Drug nano-carrier
Systemic
Laser pulse
Local
X-rays
Local
Cancer
Nanocluster
Plasmonic nanobubble
Explosion
Drug
X-rays
Therapeutic synergy
Cell death



Quadrapeutics treatment of HNSCC in mouse models