THE UNIVERSITY OF RHODE ISLAND

Formulation and Delivery of Lipophilic Drugs to Cancer Cells by pHLIP® Coated Liposomes

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THINK BIG WE DO℠

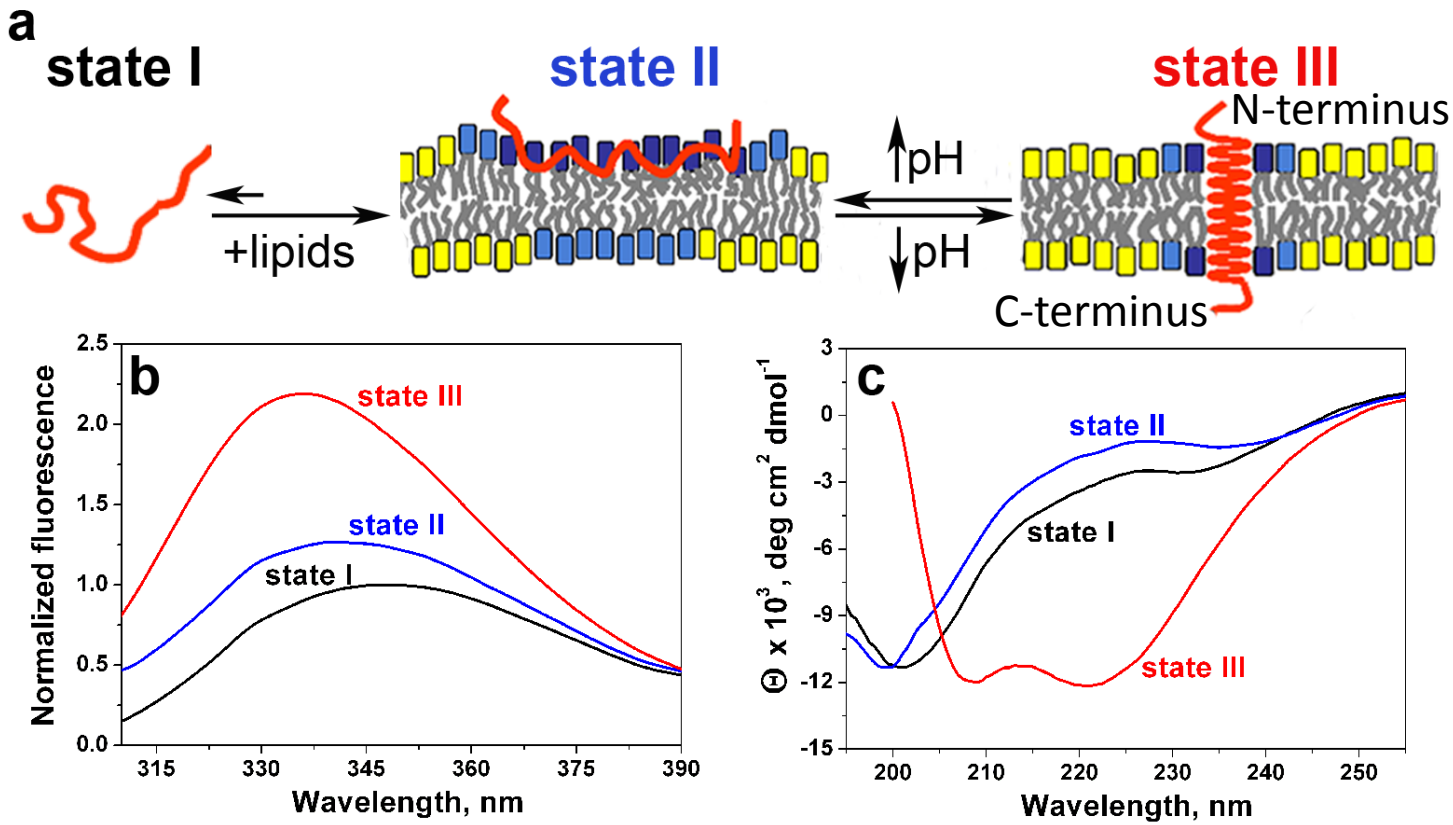
A Hallmark for Primary and Metastatic Cancer

- Cancer has reprogrammed energy metabolism
- As a result of that, cancer cells have reverse pH gradient and therefore the tumor microenvironment is acidic
- The acidity is vital for the survival of cancer cells and their proliferation
- This acidity affect adversely for the therapeutic effects of some conventional drugs and create the drug resistance

- ❑ **Can this extracellular acidity be a universal targeting method?**
- ❑ **And how can we use that?**

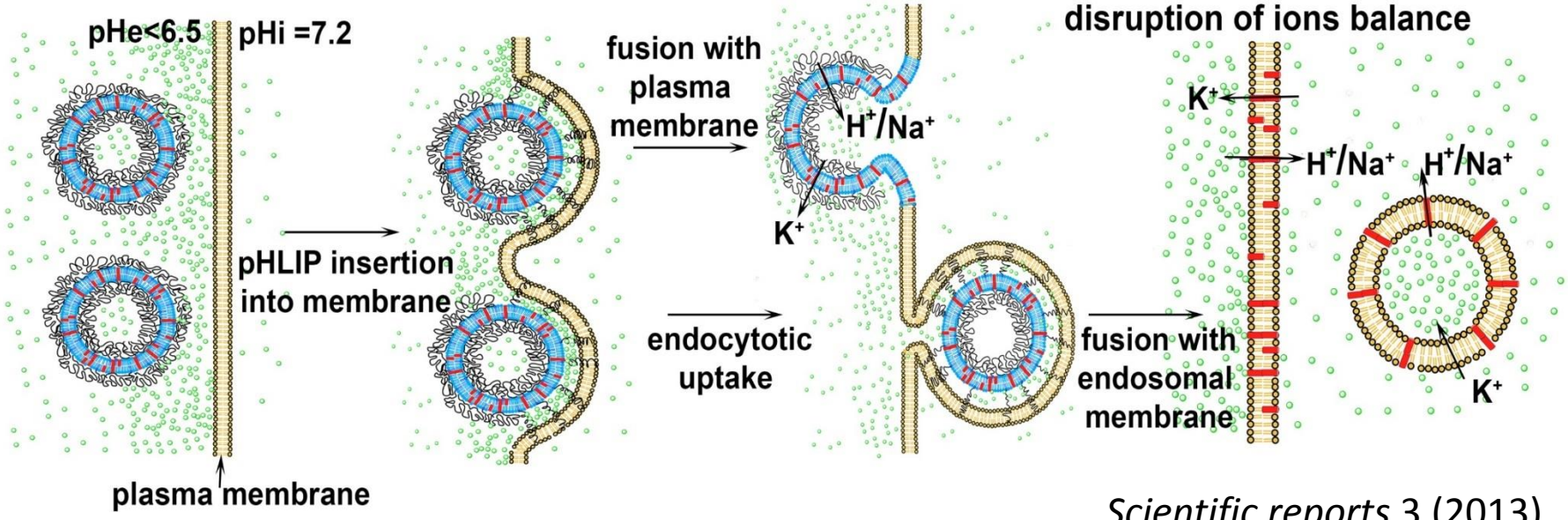
pHLIP – pH (Low) Insertion Peptide

G G E Q N P I Y W A R Y A D W L F T T P L L L L D L A L L V D A D E G T C G



- Trp fluorescence and CD are used to monitor pHLIP interaction with membrane
- pHLIP targets acidity!

Our goal: pH dependent transfer of nano-pores into membrane of cancer cells to induce apoptosis



Scientific reports 3 (2013).

- **Proper balance of ions in intracellular and extracellular space is the key for normal cell functioning**
- **Changes in the conductance of membranes for ions will lead to cell death**

Gramicidin

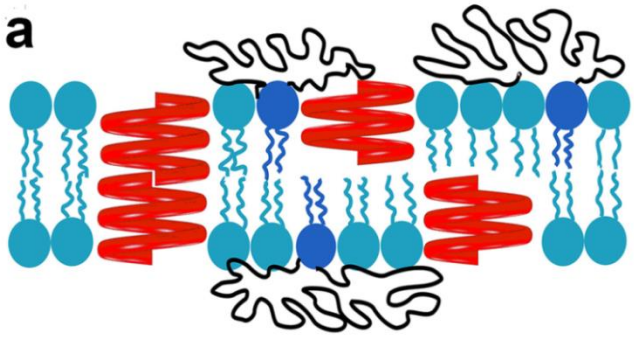
formyl-(L)X-(D)Gly-(L)Ala-(D)Leu-(L)Ala-(D)Val-(L)Val-(D)Val-(L)Trp-(D)Leu-(L)Y-(D)Leu-(L)Trp-(D)Leu-(L)Trp-ethanolamine

X = Valine OR Isoleusine

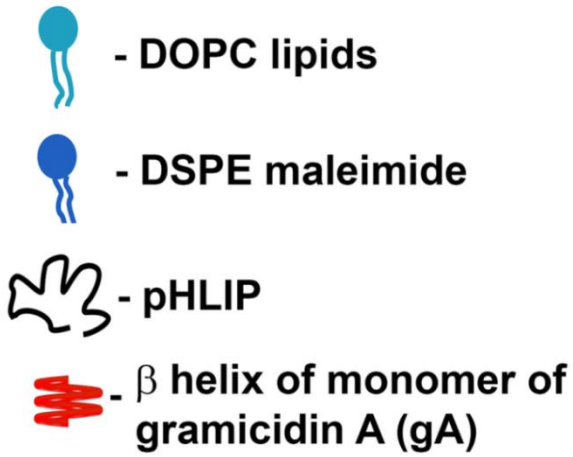
If Y= tryptophan Gramicidin A
If Y= phenylalanine Gramicidin B
If Y= tyrosine Gramicidin C

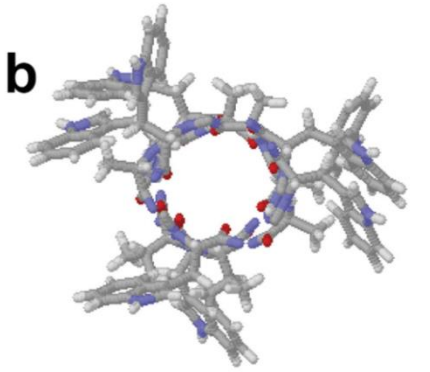
- **An antibiotic obtained from the bacterial species *Bacillus brevis***
- **It's effective against gram-positive bacteria**
- **It cannot be administered internally**

Gramicidin A ion pores



lipid bilayer of pHLIP-coated liposomes containing gA





top view of ion-conductive gA helix

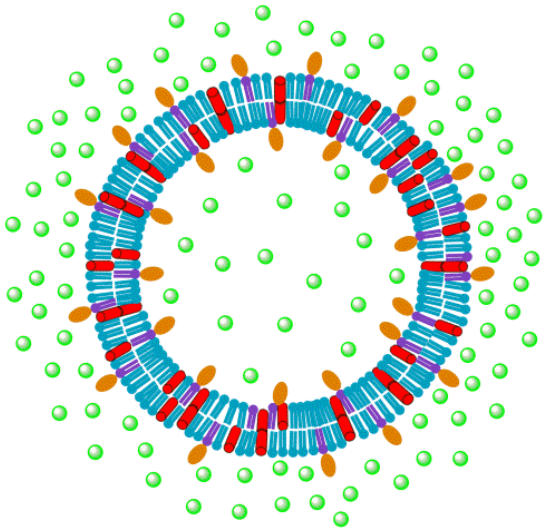
- Acts as On/Off switch
- Pore Diameter is 4-5 Å
- Ion transfer rate is ~ 10^7 cations per second

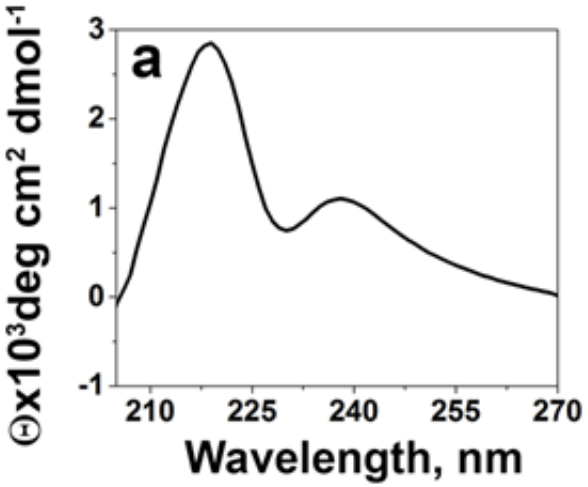
Biophysical Data

mol ratio

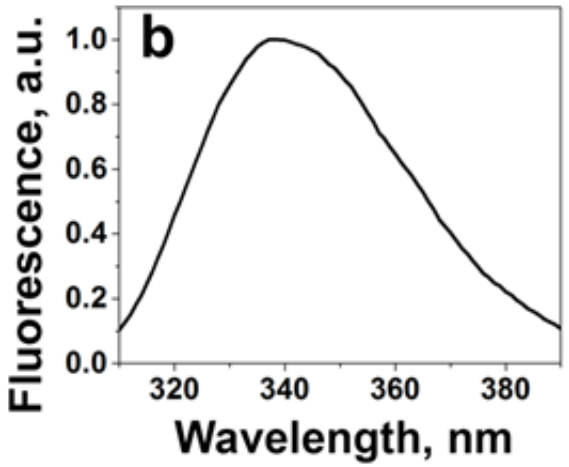
DOPC : DSPE-pHLIP : gA
95 : 5 : 10

gA forms channels in the membrane

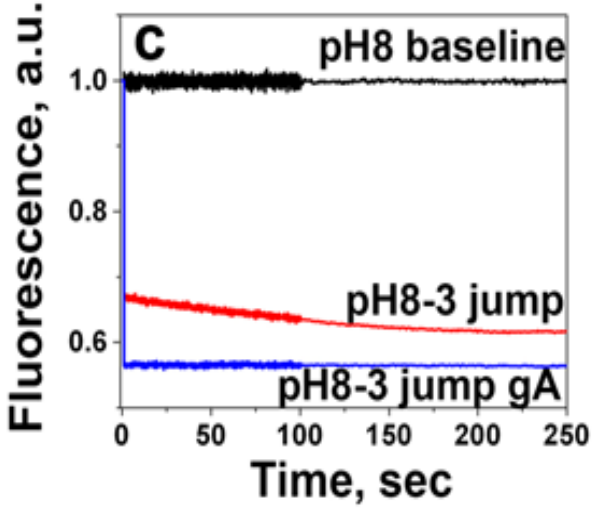




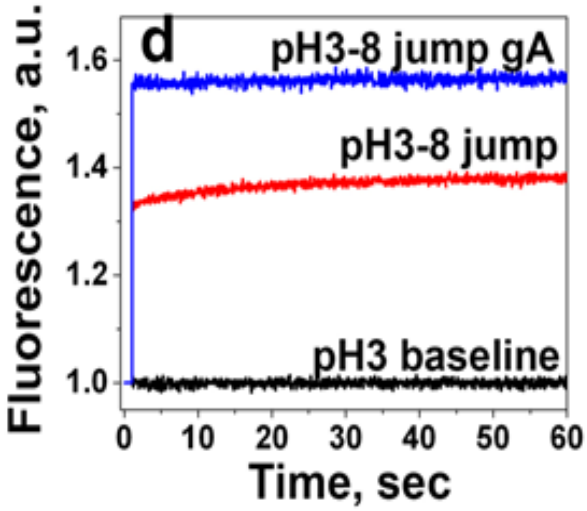
CD



Trp Fluorescence

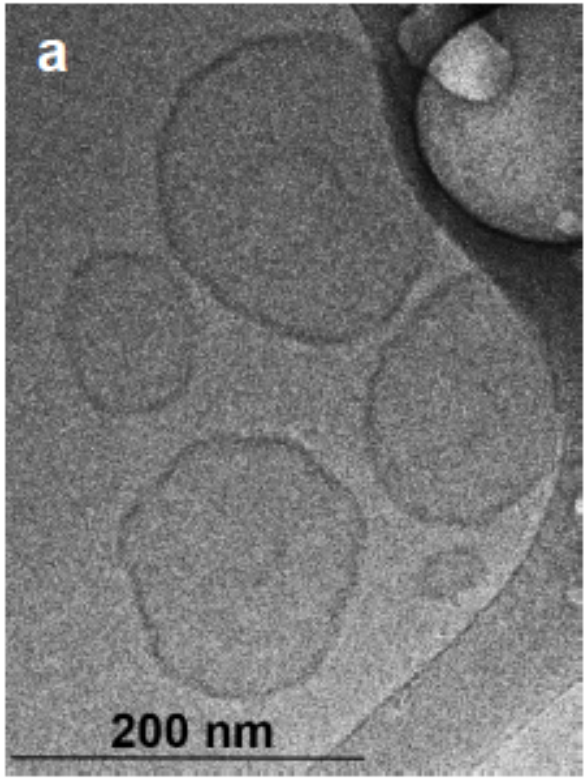


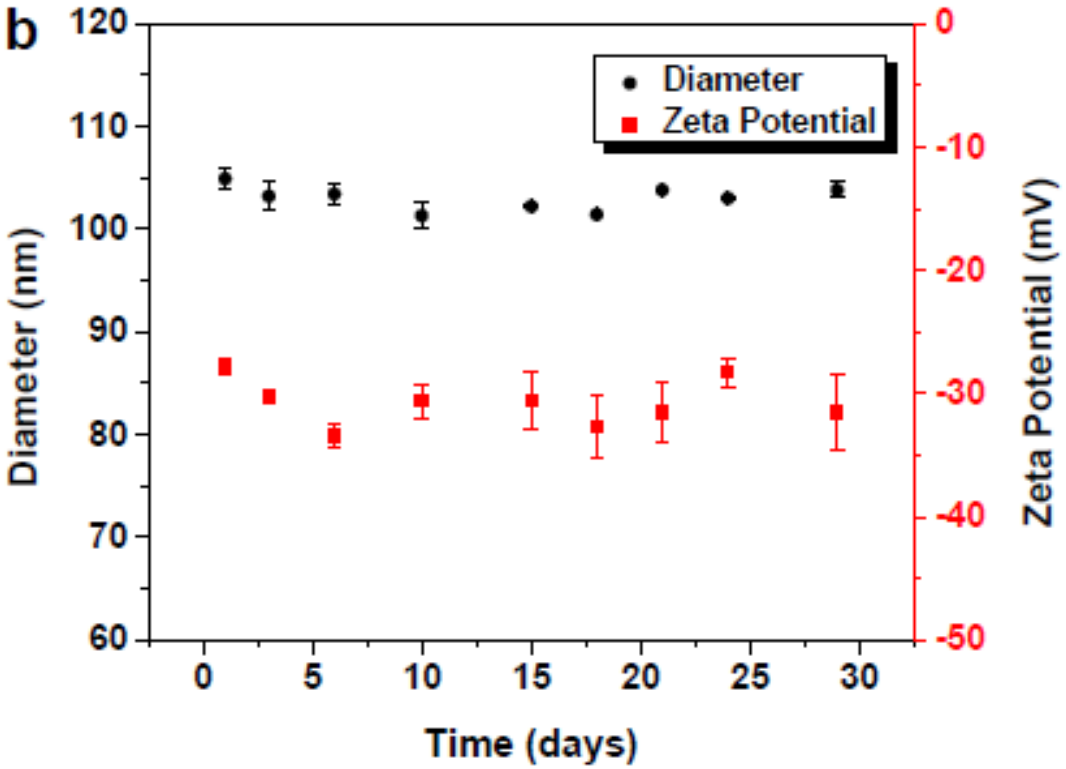
FITC-quenching



FITC-dequenching

Stability

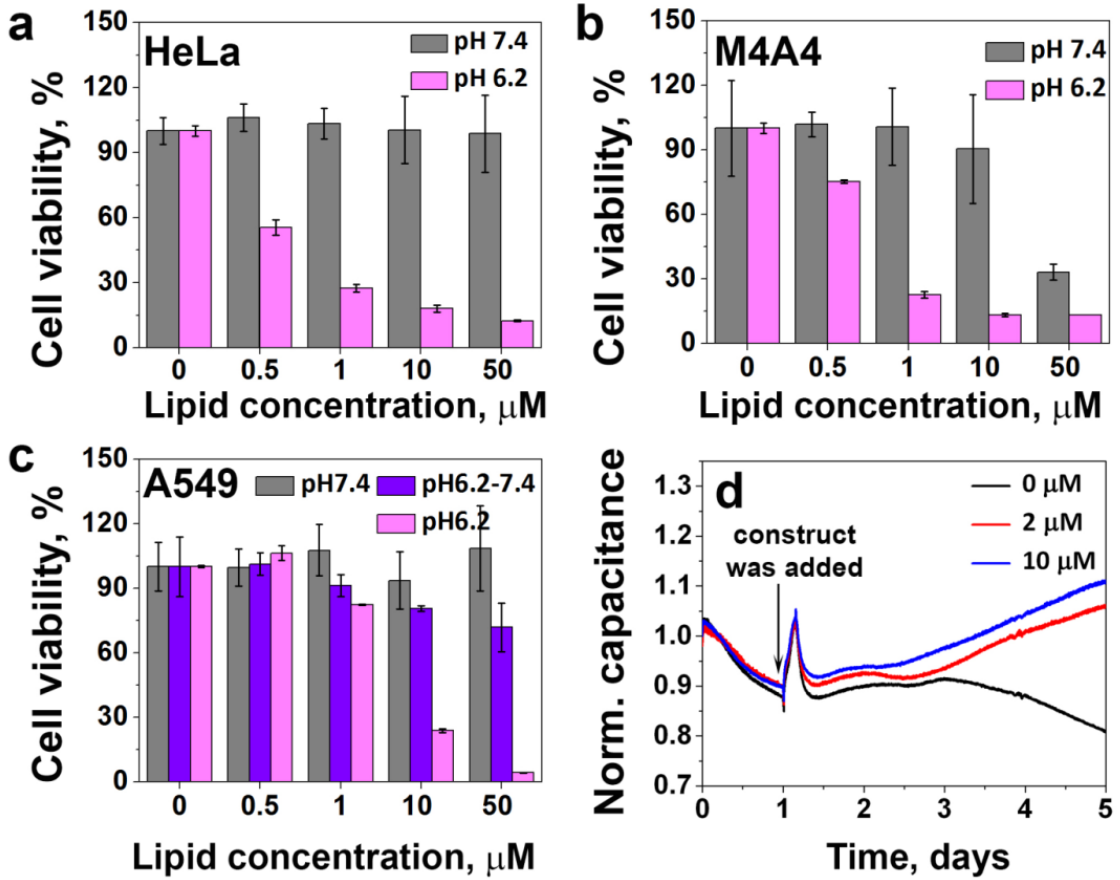




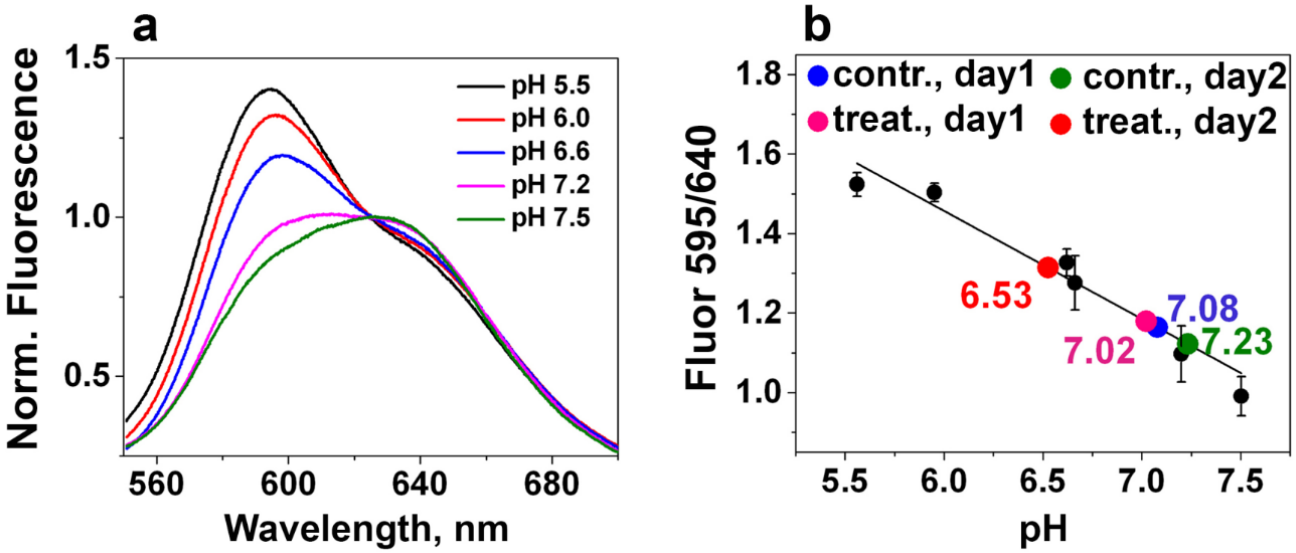
- Average diameter ~ 105 nm (PDI 0.07 ± 0.01)
- Average zeta potential ~- 30 mV
- pHLIP enhanced the stability of vesicles and gives them longer shelf life at 4 ℃

In-vitro :MTS and ECIS

- Cell proliferation assays show pH and concentration dependent toxicity for cells
- Kinetics show that the cell death starts at ~ 1-2 days from the treatment



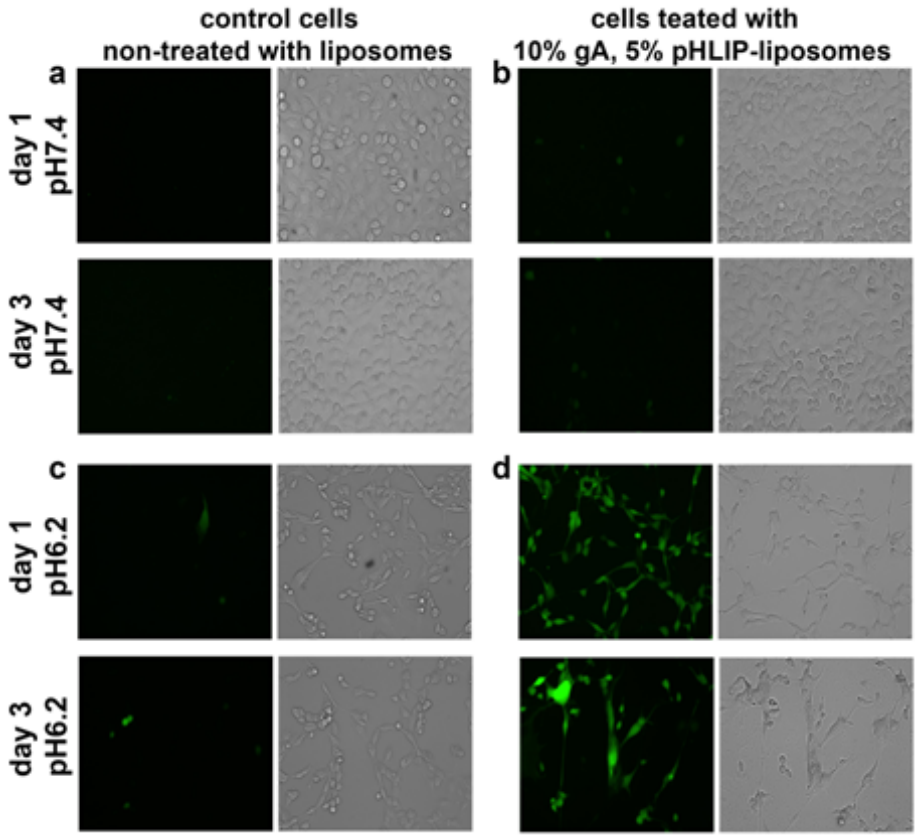
Changes of interacellular pH

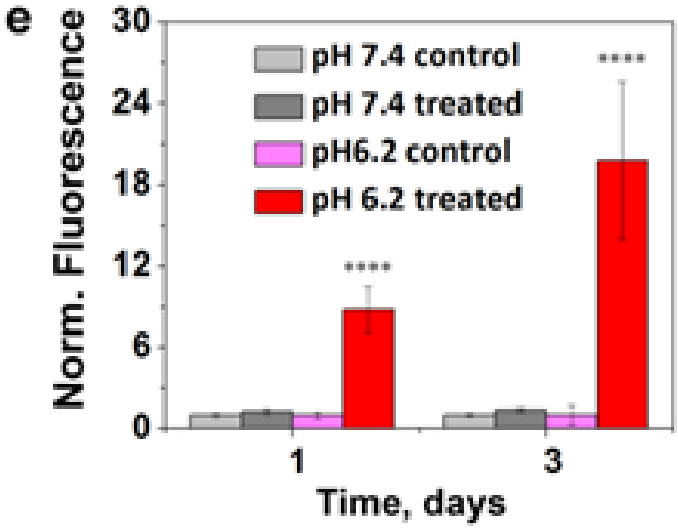


Day1: pHi of non treated and treated cells were 7.08 and 7.02 respectively

Day2: pHi of treated was 6.53 where as in non treated it remained at neutral pH

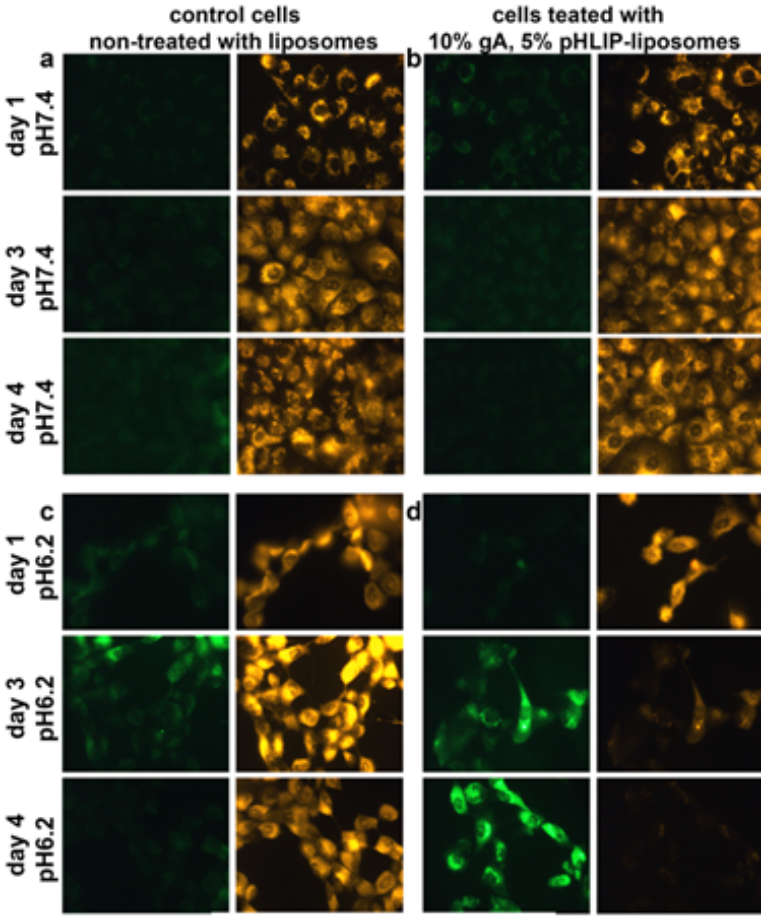
Changes of intracellular Na^+ concentration

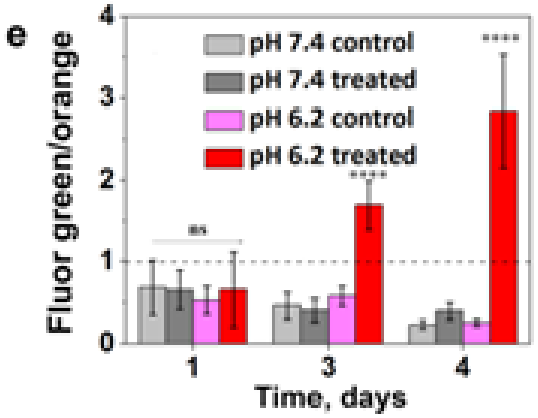




- Cells treated with liposomes at low pH showed enhancement in green fluorescence from Corona Green indicator on day 1 and even more on day 3

Mitochondria depolarization assay





- Apoptosis of cells can be monitored by depolarization of mitochondria
- JC-9 - exhibits potential-dependent accumulation in mitochondria
- Only cells treated at low pH showed increase in green/orange fluorescence ratio from third day – mitochondria depolarization and cell apoptosis.

Summary

- Acidic tumor microenvironment is a hallmark of many forms of cancerous tumors.
- pHLIP technology selectively targets the acidic tissue
- The pHLIP-coated liposomes can deliver the gramicidin channels to the cellular membrane of cancer cells which induce disbalance of monovalent cations following by mitochondria depolarization and apoptosis.
- The pHLIP coated liposomes can be used to deliver of various membrane peptides , proteins and hydrophobic drugs such as paclitaxel.

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