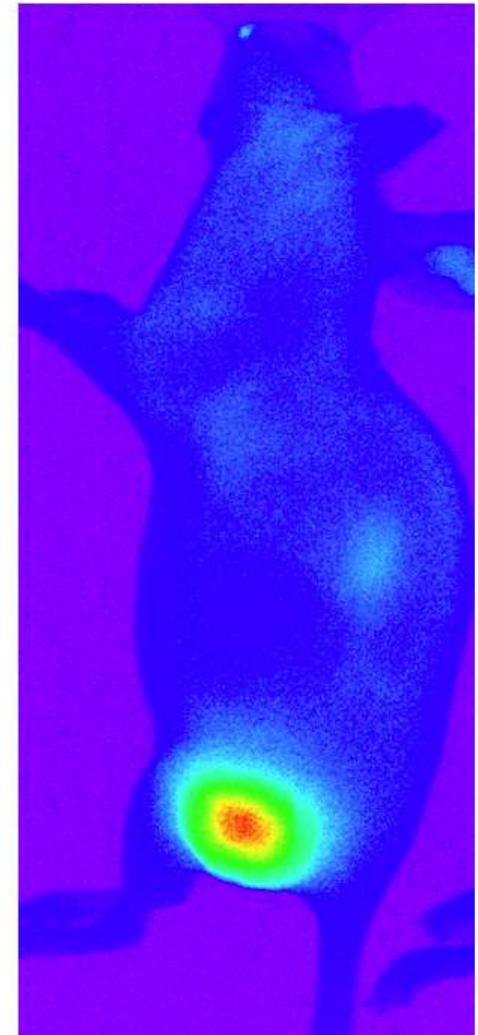


Methods of detection and delivery of therapeutics to malignant tumors.

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Outline

- **Direct placement methods**
 - wafers, particles, thermogels...
- **Targeted delivery**
 - antibody-drug conjugates
 - penetrating peptides
 - pHLIP
- **Conclusions**

Anticancer Drugs and Side Effects

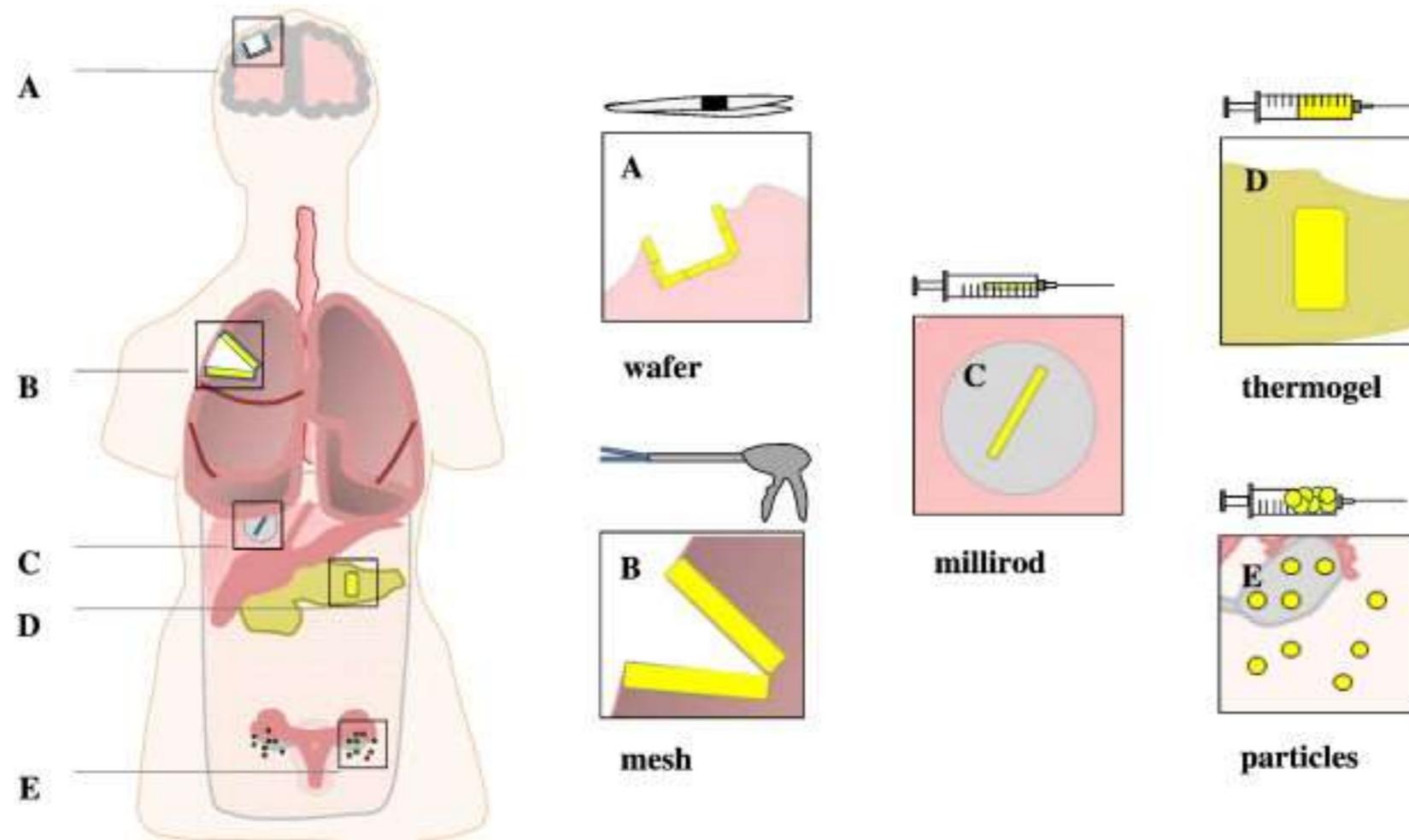
- Many anticancer drugs have off-target side effects.
- Severely limit the efficacy of chemotherapy.

Site of toxicity	Cyclophosphamide	Methotrexate	Fluorouracil	Vincristine	Doxorubicin
Bone marrow	■ ■	■ ■ ■ ■	■ ■ ■ ■	■	■ ■ ■ ■
Digestive track	■ ■	■ ■	■ ■	■	■ ■
Skin, hair	■ ■	■	■	■ ■	■ ■
Heart					■ ■ ■ ■
Kidney		■ ■		■	
Liver	■	■ ■			
Nervous System			■	■ ■ ■ ■	
Lung	■	■			

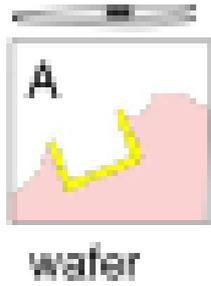
■ occasional ■ ■ common ■ ■ ■ ■ severe, dose-limiting

○ Clear needs for targeted therapies

One way to circumvent side effects: place drugs locally

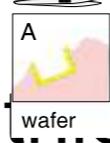


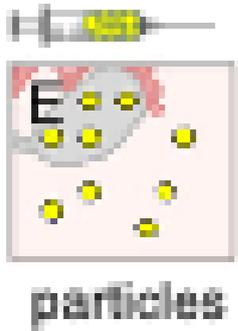
A and B are in clinical use, others are in different stages of pre-clinical and clinical trials



Gliadel Wafer

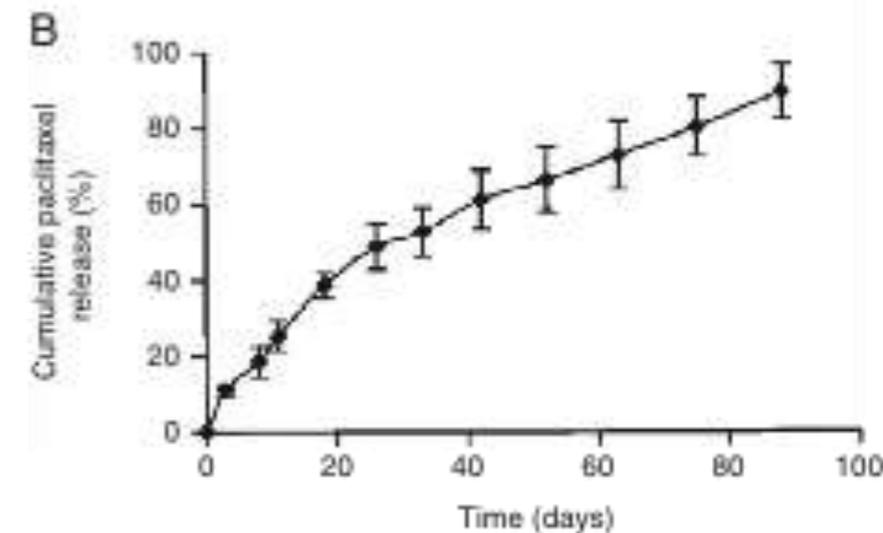
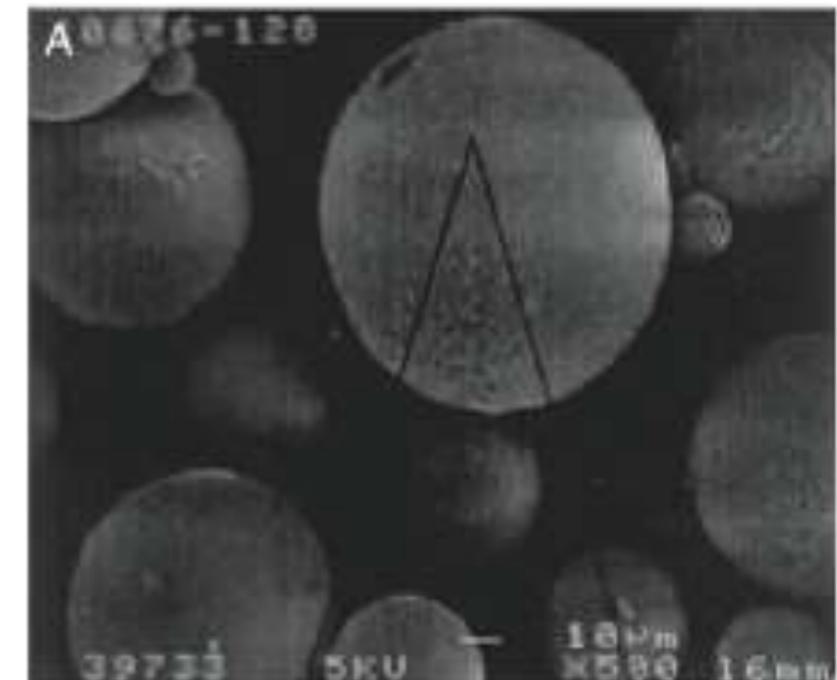


- Most well-studied and successful drug delivery implant for treatment of recurrent brain cancer (glioma).
- 14mm in diameter, 1mm k (size of a dime).
- contains chemotherapeutic *carmustine*
- placed in the tumor resection cavity (up to 8 wafers)
- biodegradable polymer (gone after 6-8 weeks)
- releases the drug over time (5 days)



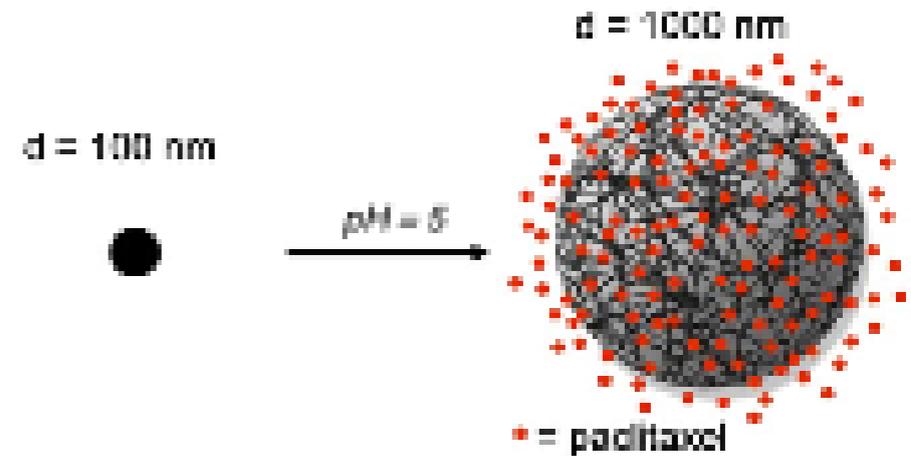
Paclimer Microspheres

- Intended for intraperitoneal administration and prevention of recurrent ovarian cancer.
- Contains drug *Paclitaxel*
- Average diameter: 53 μm
- Range: 20-200 μm
- Rate of drug release: 1-2%/day over 90 days.
- In Phase I clinical trial.



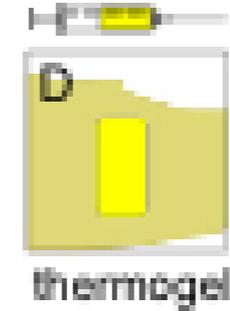


Nanoparticles

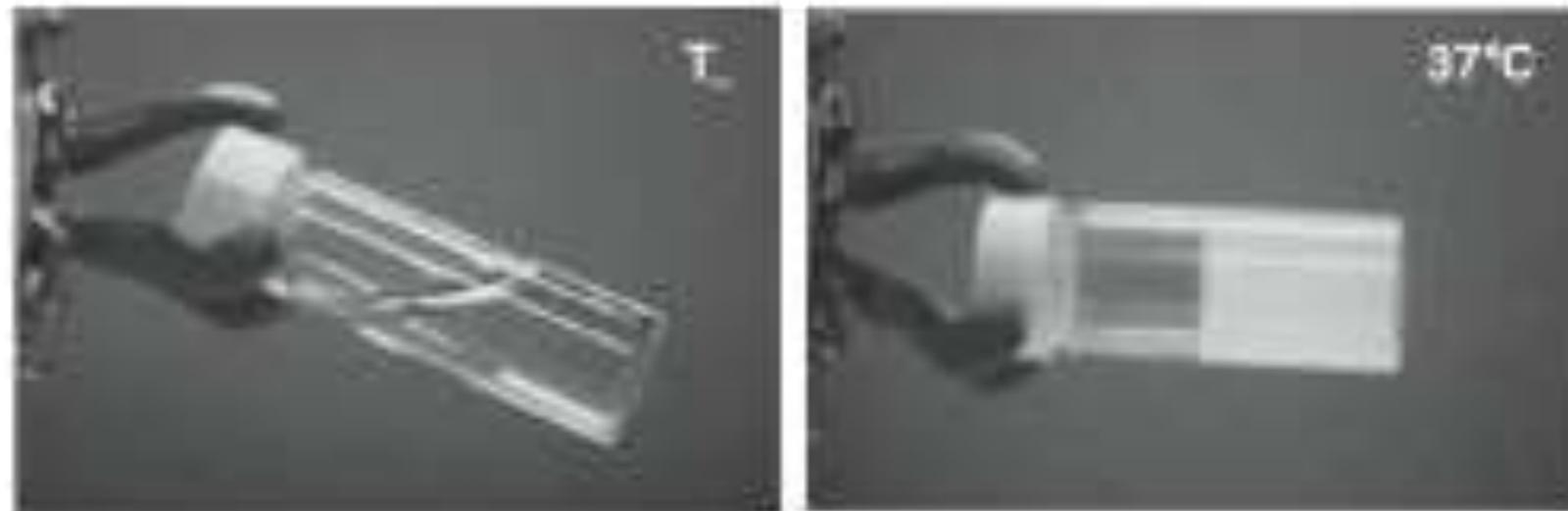


- When taken up by the cells, end up in the lysosome (where pH ~ 5) Under acidic pH conditions, swell 10x and release their drug.
- Their utility has been demonstrated in two cancer models in rodents: lung and mesothelioma.
- Have not been tested in humans.

Thermogels



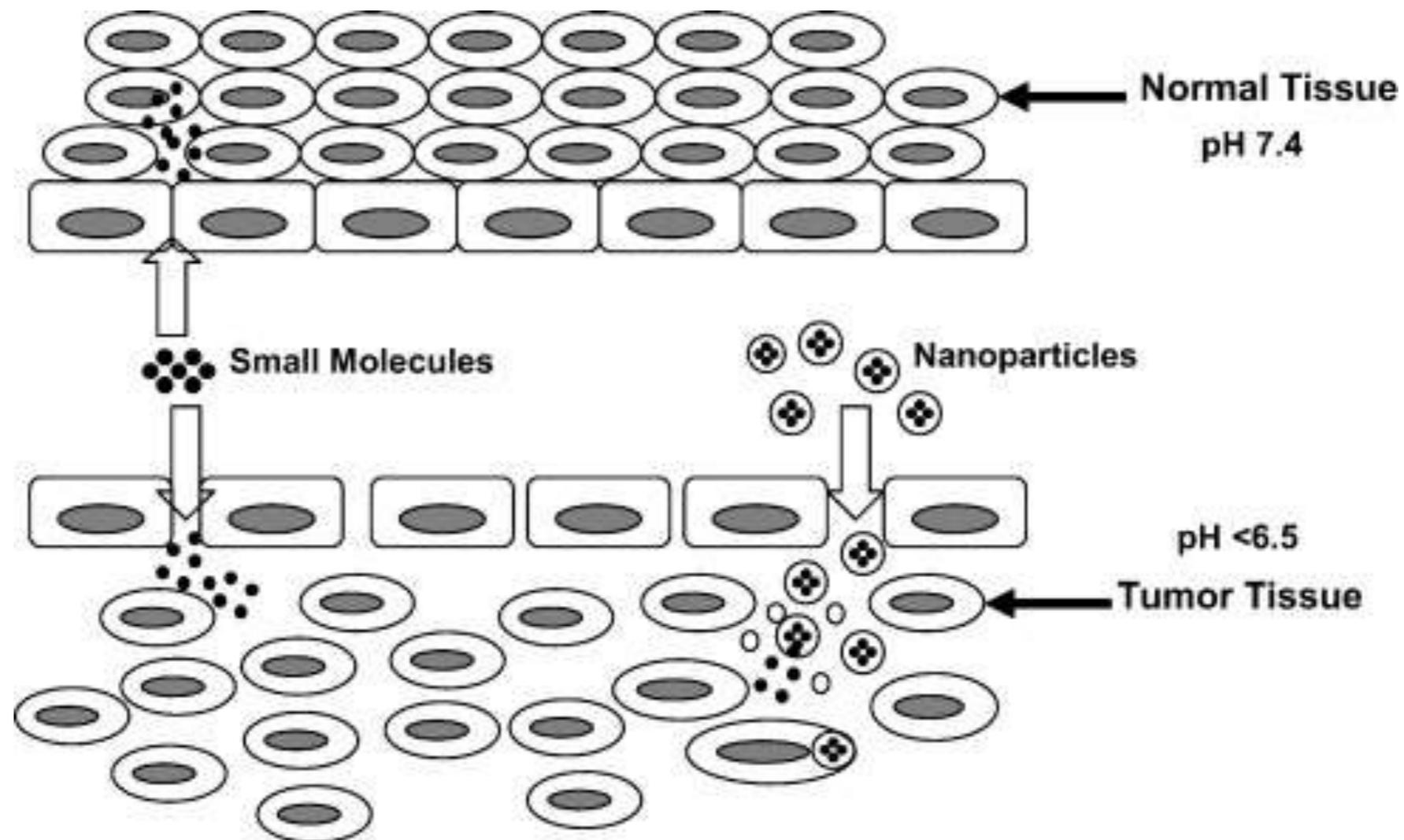
- Eg: Chitosan hydrogel
- Made from chitin (found in the shells of crustaceans - eg. crabs and shrimp)
- biodegradable and biocompatible
- can deliver chemotherapy or radiation
- Liquid at room temperature - gels at body temperature
- pre-clinical studies demonstrate efficacy against solid tumors in mice.



**What about “long distance”
targeting of tumors?**

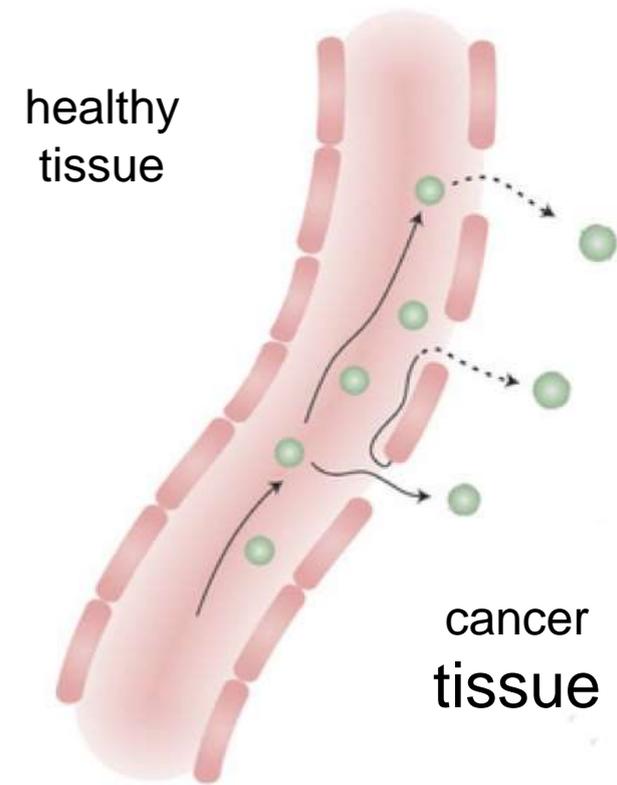
Some tumor hallmarks

- Increased permeation
- Decreased pH



Drug Carrier Systems

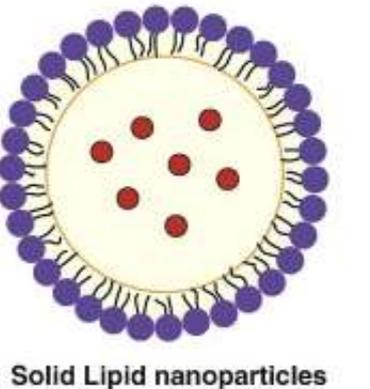
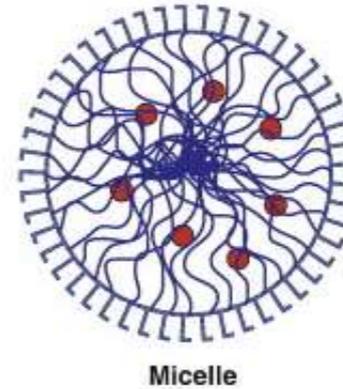
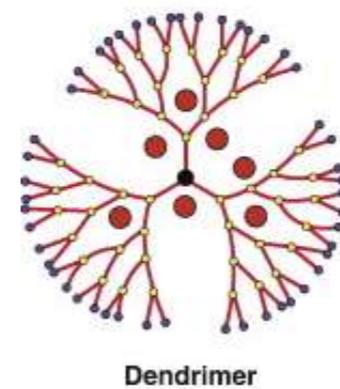
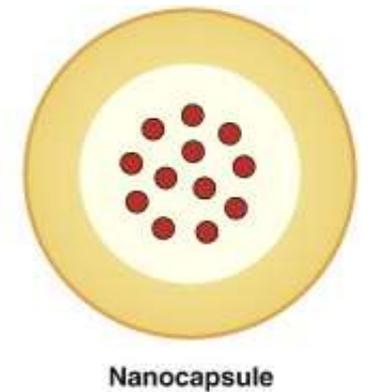
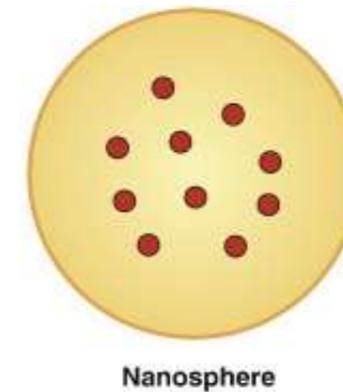
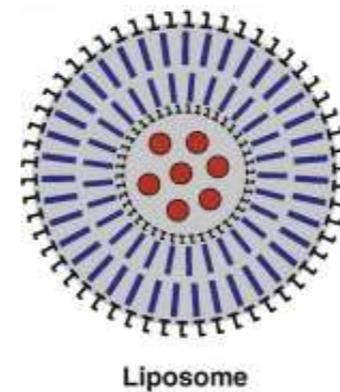
- Can improve the therapeutic index by reducing :
 - * Side effects in healthy tissues.
 - * The overall dose by concentrating the drug in the targeted tissue.



- Carrier systems include:

- Passively target tumors due to the increased permeation of many solid tumors.

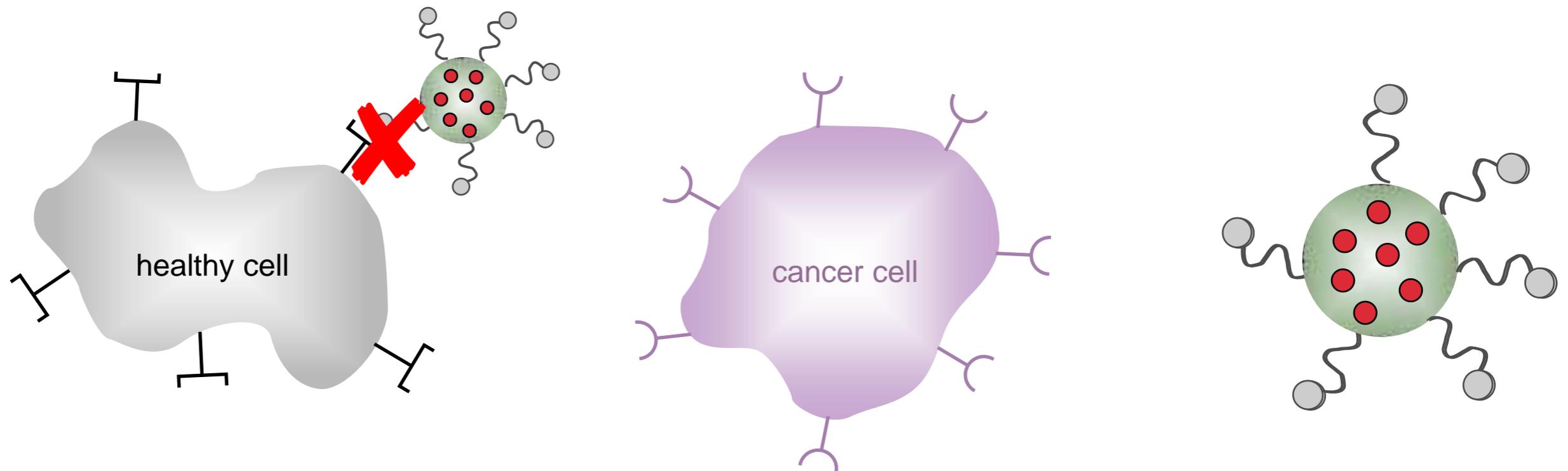
- However, this effect is small for certain tumors.



○ Other more specific strategies have been developed

Targeting Strategies

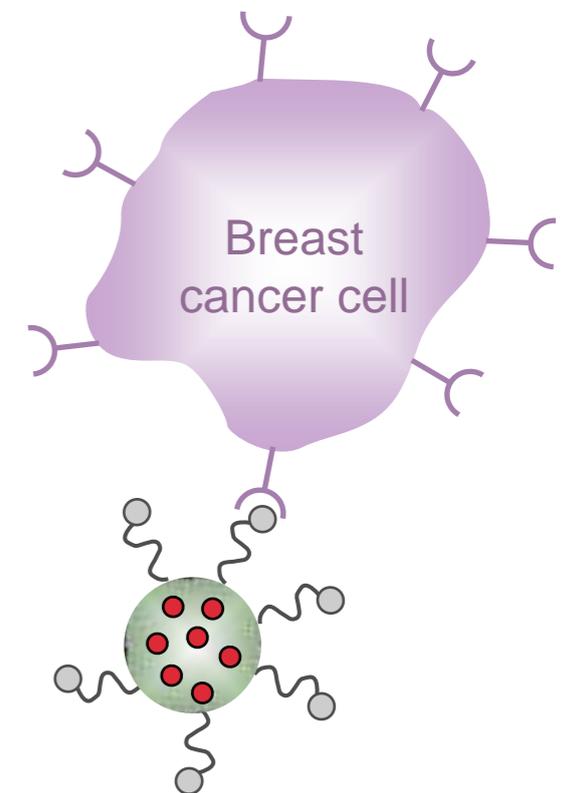
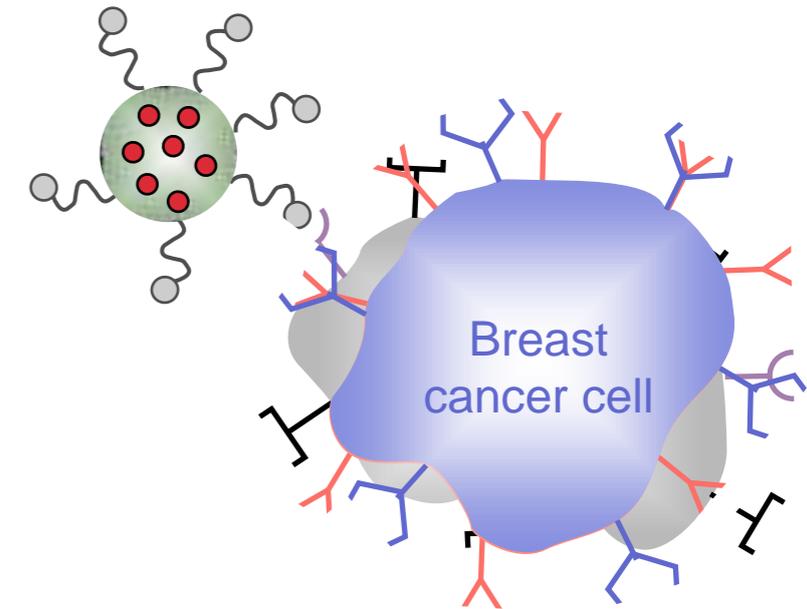
- Most take aim at specific cancer cell surface biomarkers.
 - * Example: over-expressed cell surface receptors.
 - * Involve the addition of ligands to the carrier system.



○ Allows specific interaction with cancer cells

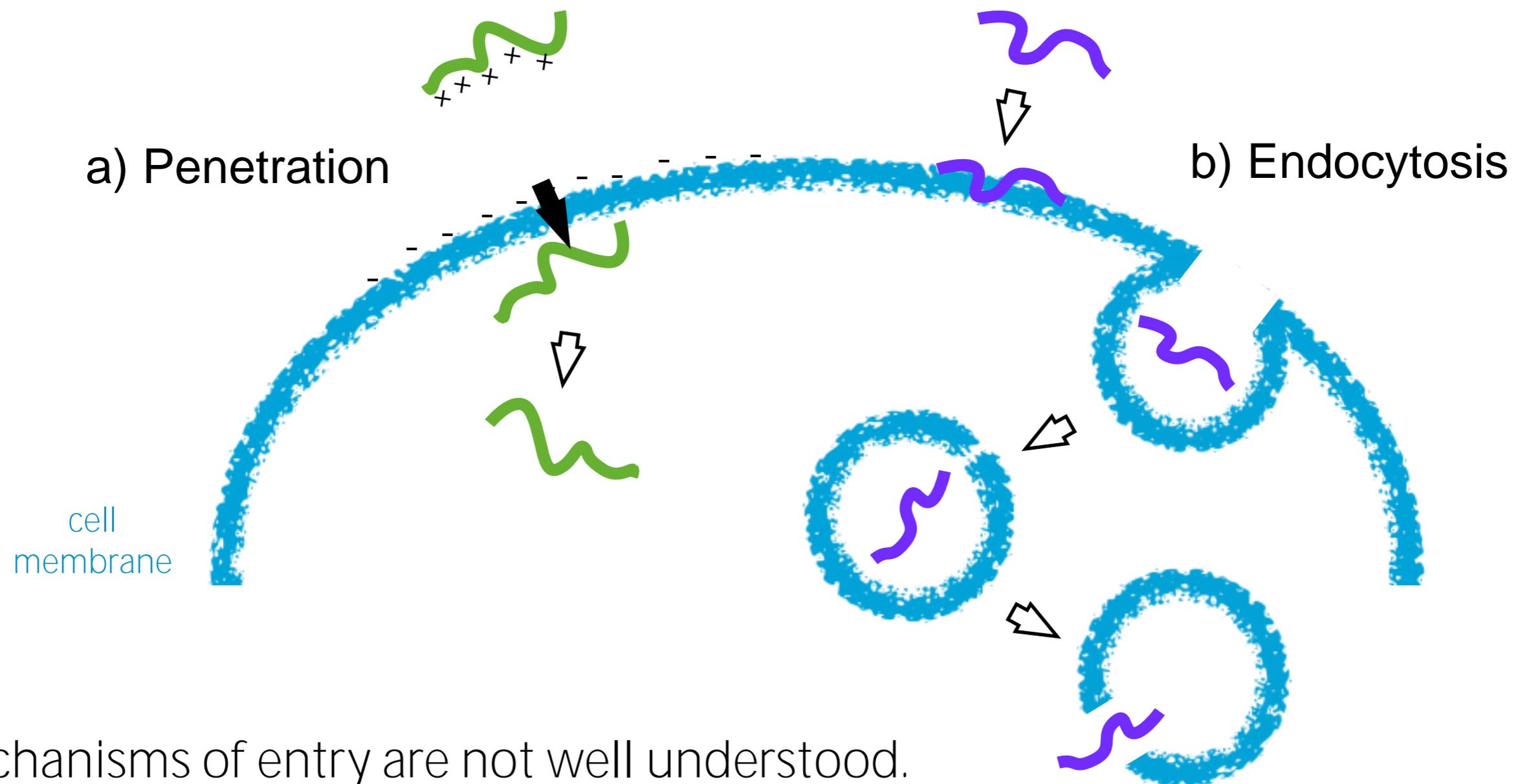
Disadvantages of targeting biomarkers (like cell surface receptors)

1. Healthy cells also have the same biomarkers.
 - * In lower number than in cancer cells.
 - Targeting system also interact with healthy cells.
2. Different cancers have different biomarkers.
 - Design of specific ligand for each type of cancer.
3. Even in the same tumor, cancer cells can have different biomarkers.
4. These systems still need to be transferred into the cells.
 - Need for a delivering “device”



Delivering Devices: Cell Penetrating Peptides

- Most are small positively charged piece of protein (peptide).
- Bind to the negative charges present on the surface of cells.

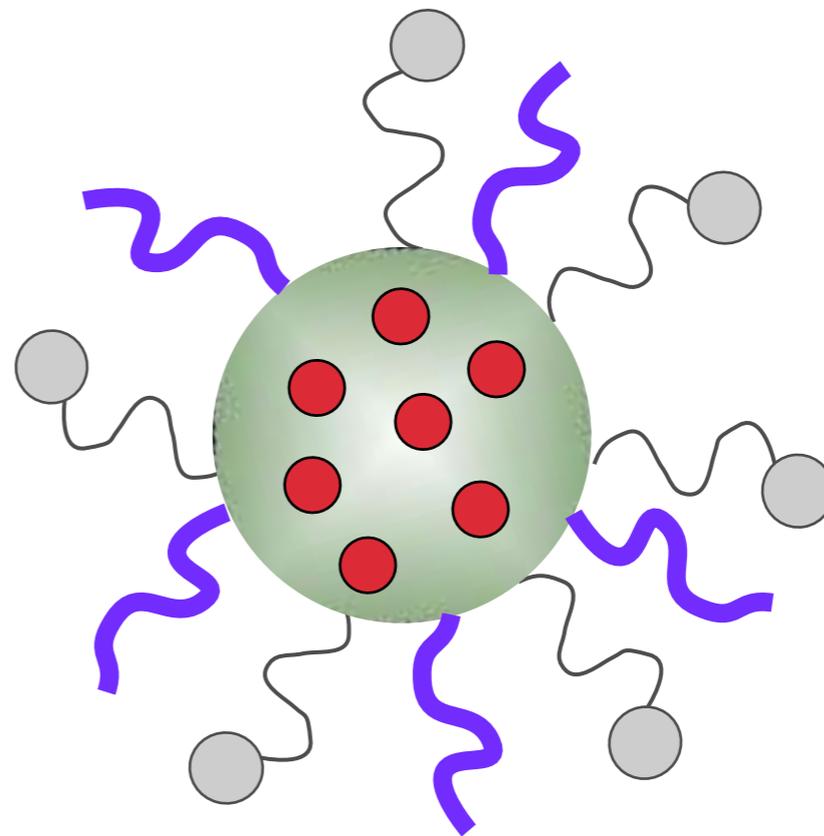


- Mechanisms of entry are not well understood.

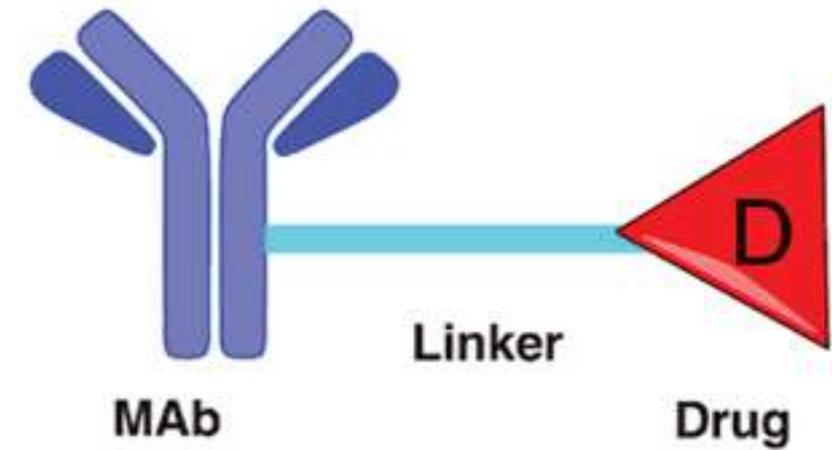
Existing Targeting and Delivering Systems

They are often big and made up of many components:

1. Carrier
2. Drug
3. Specific ligand
4. Penetrating peptide



Another example of biomarker targeting: Antibody-drug conjugates (ADCs)



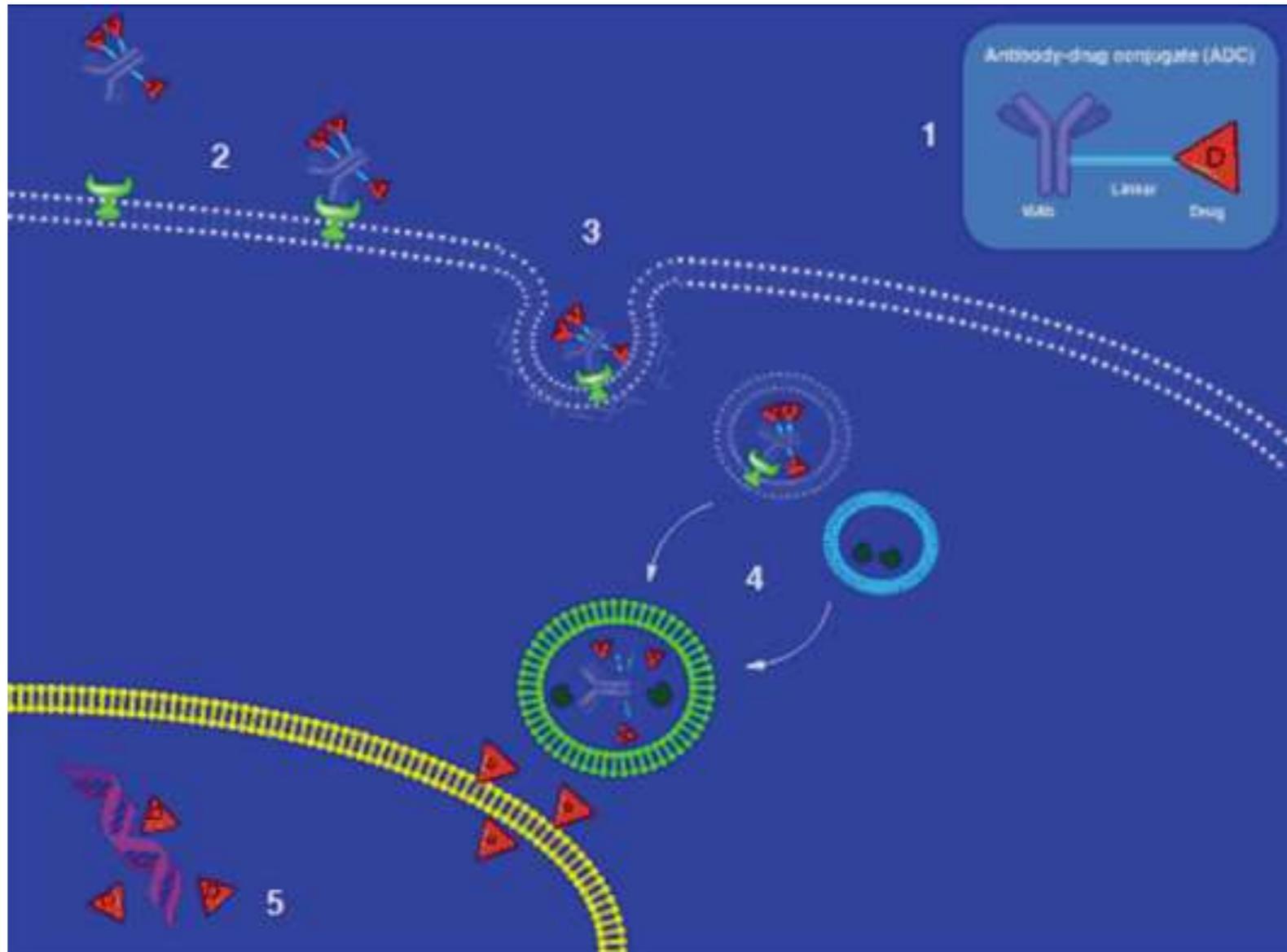
Delivery Device: Antibody

Requirements:

- A target antigen, which is cancer specific
- An antibody that displays a high binding affinity to the antigen
- A cytotoxic agent that is highly potent (sometimes more than 1)
- A linker designed to allow ADCs to remain inactive when in the blood

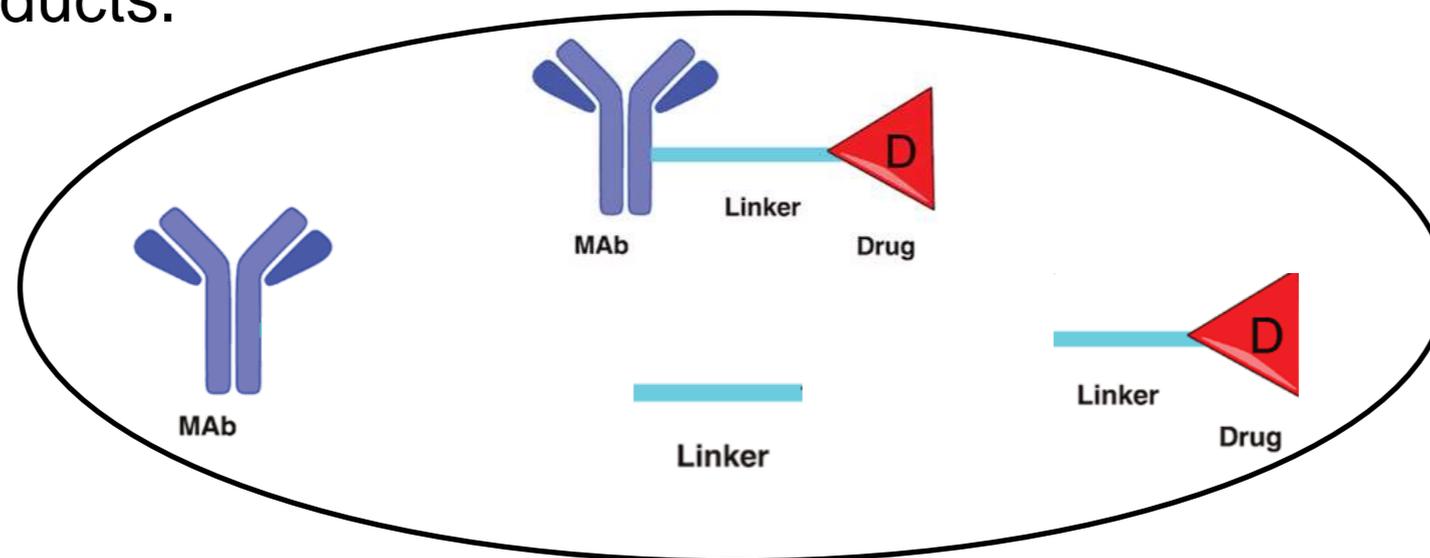
How do ADCs work?

- The ADC is delivered intravenously and localizes to target tumor cells by binding to tumor specific antigens.
- Internalization of the ADC.
- Proteases and hydrolases digest the antibody and linker to release free drug.
- The drug then binds to its molecular target, leading to cell death.



Difficulties with ADCs

- Many ADCs are in clinical trials (most in Phase I and II)
- Few did not pass Phase III trials
- One ADC was recently approved by FDA (February, 2013)
 - **trastuzumab emtansine - ADC against HER2-positive advanced breast cancer - improves survival by 6 months.**
- The idea behind ADCs is great, but because each component has to be carefully selected, these often fail before getting to Phase III trials.
- final ADCs are often heterogeneous: mixtures rather than clean products.



○ Need for a simpler and more general targeting system.

A peptide that senses changes in pH

state I
in solution

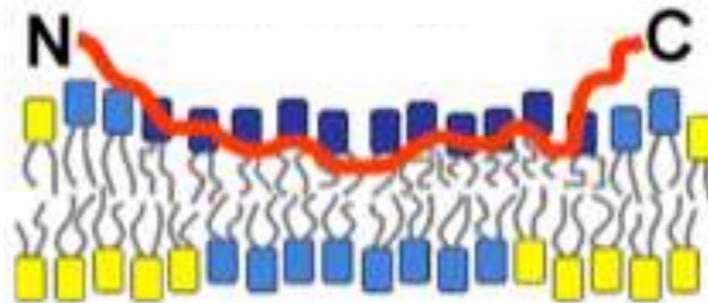


pH 8
+ lipids



A horizontal double-headed arrow with a longer right-pointing arrow, indicating a transition from state I to state II.

state II
+ cell membrane

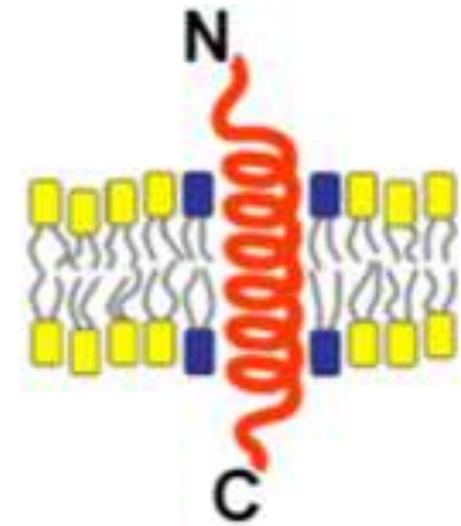


pH ~ 6



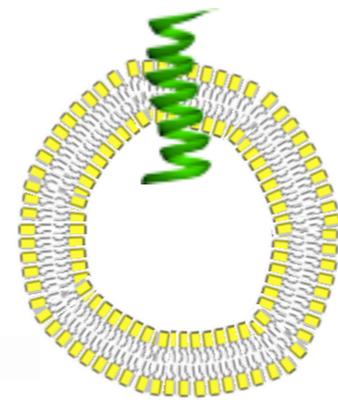
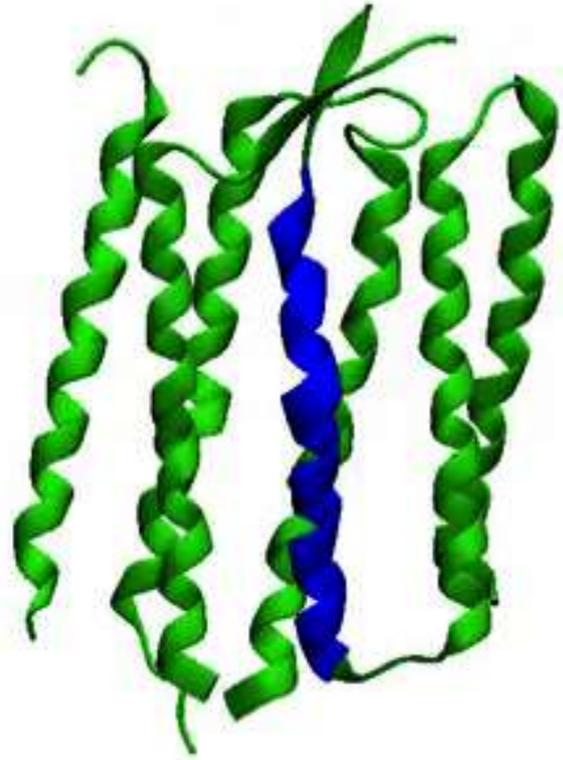
A horizontal double-headed arrow with a longer right-pointing arrow, indicating a transition from state II to state III.

state III
inserted

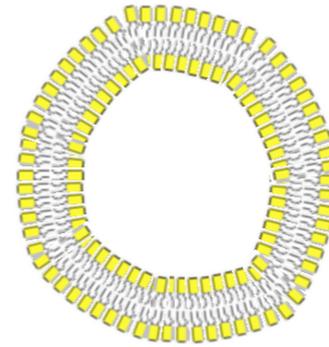


From where does this peptide come?

Bacteriorhodopsin

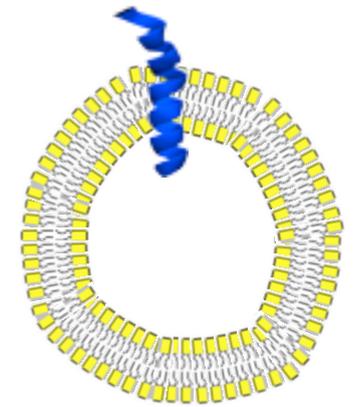


6 out 7
Transmembrane
domains



Helix C

low pH
→

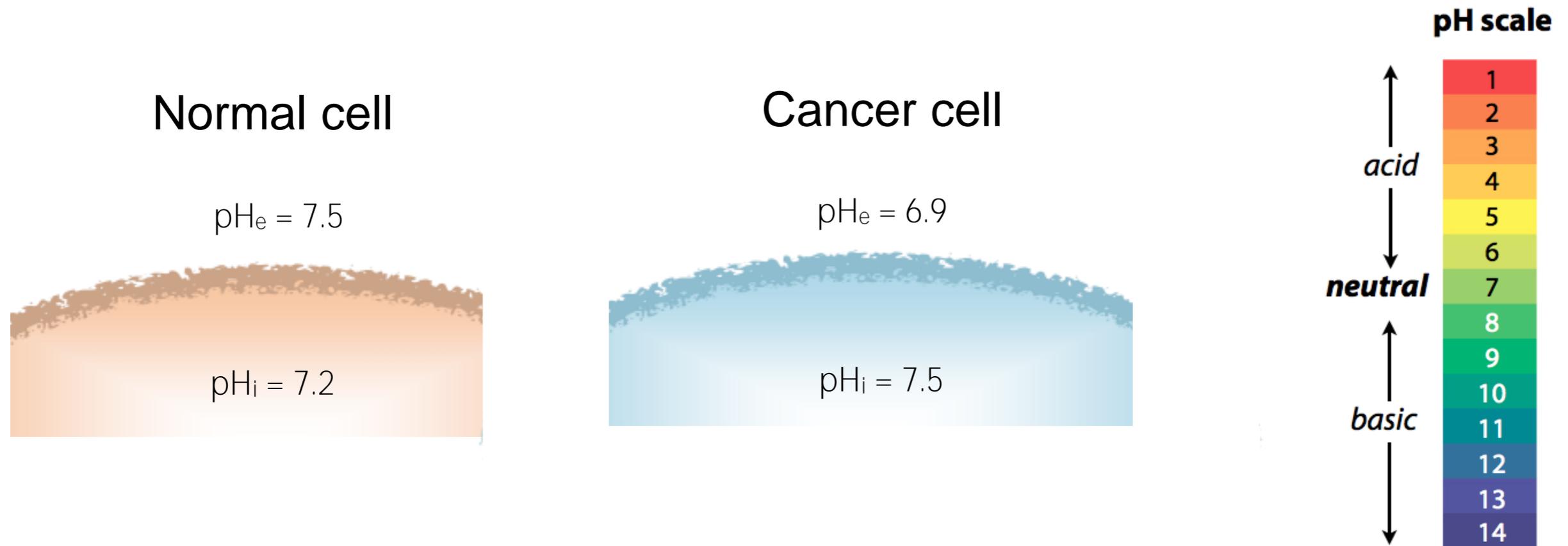


- Bacteriorhodopsin = Light-driven proton pump found in the membrane of halobacteria.
- Helix C: only one to insert spontaneously across lipid bilayers at low pH.

○ New name = pH(Low) Insertion Peptide or pHLIP

Why is low pH interesting?

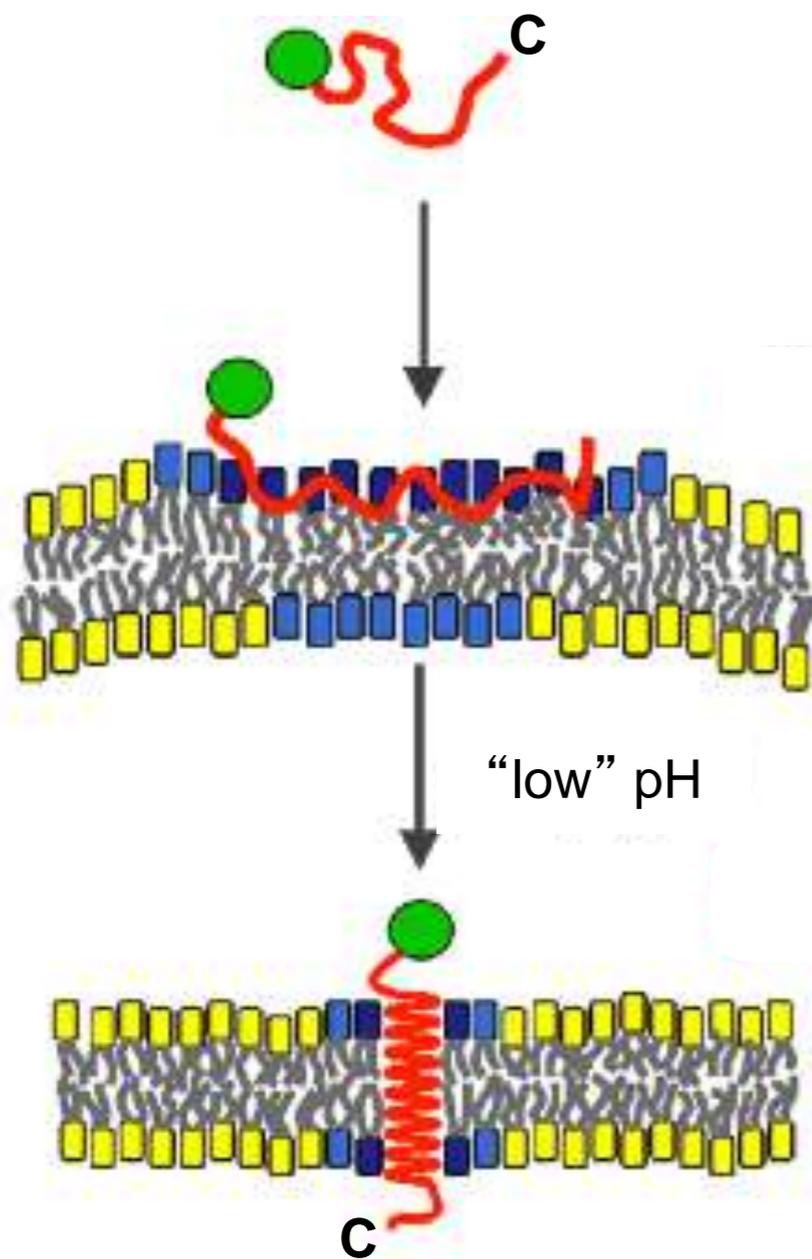
- Cancer cells have a micro-environment different from surrounding normal tissues. Tumors are characterized by a lower extracellular pH (acidic) when compared to healthy tissues:



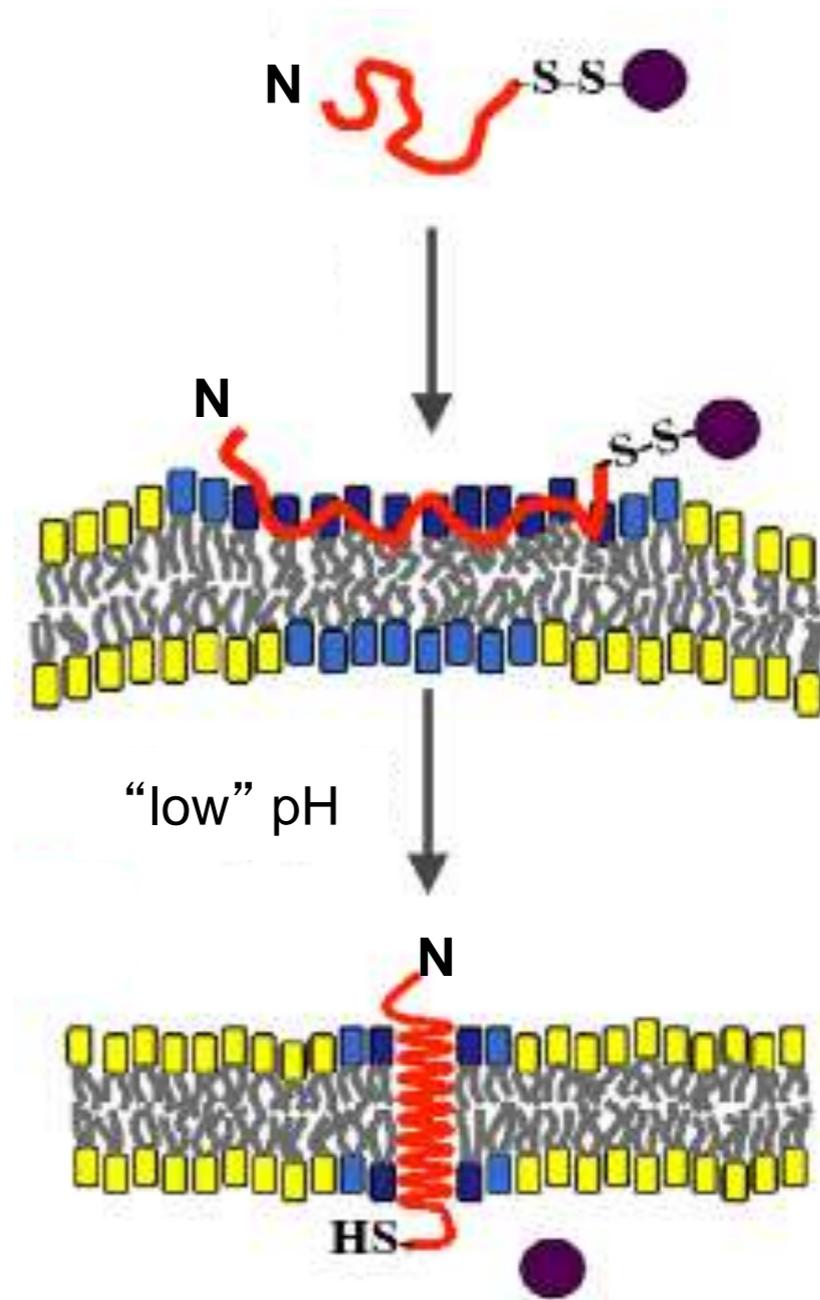
○ Acidosis = general biomarker of tumors.

Opportunities

Imaging



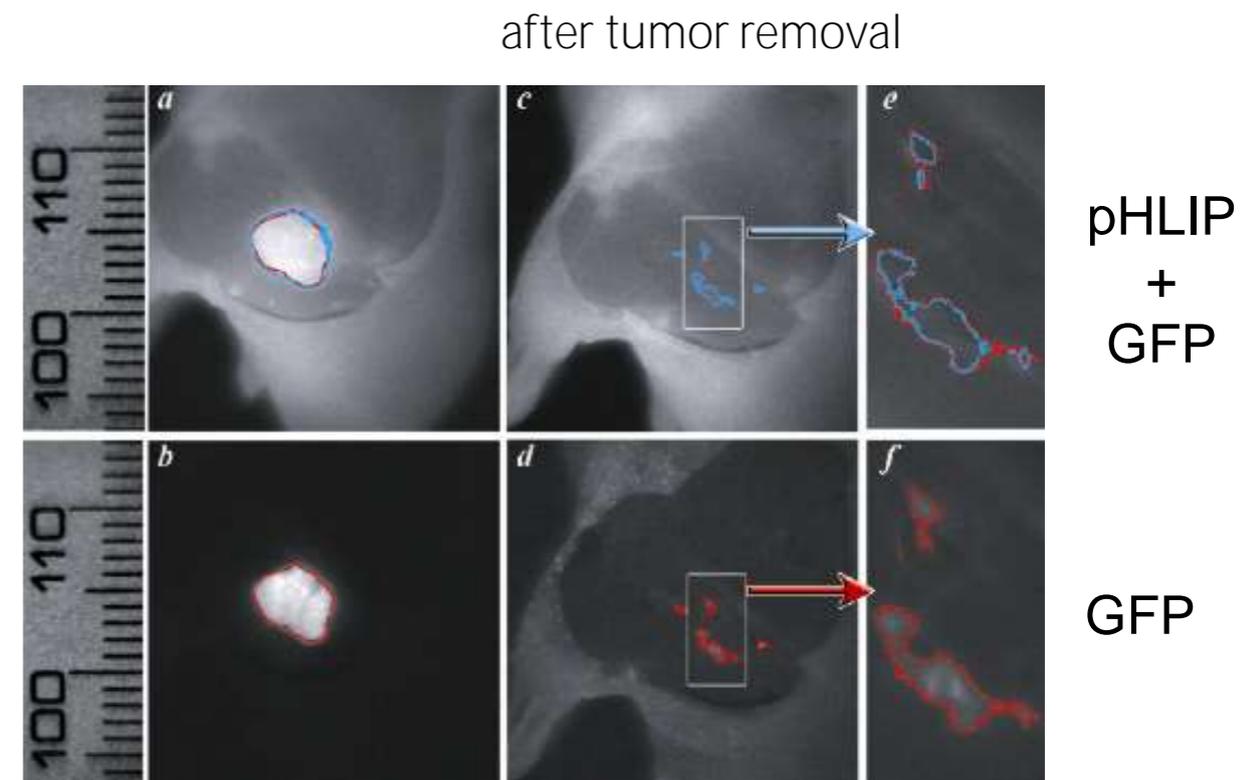
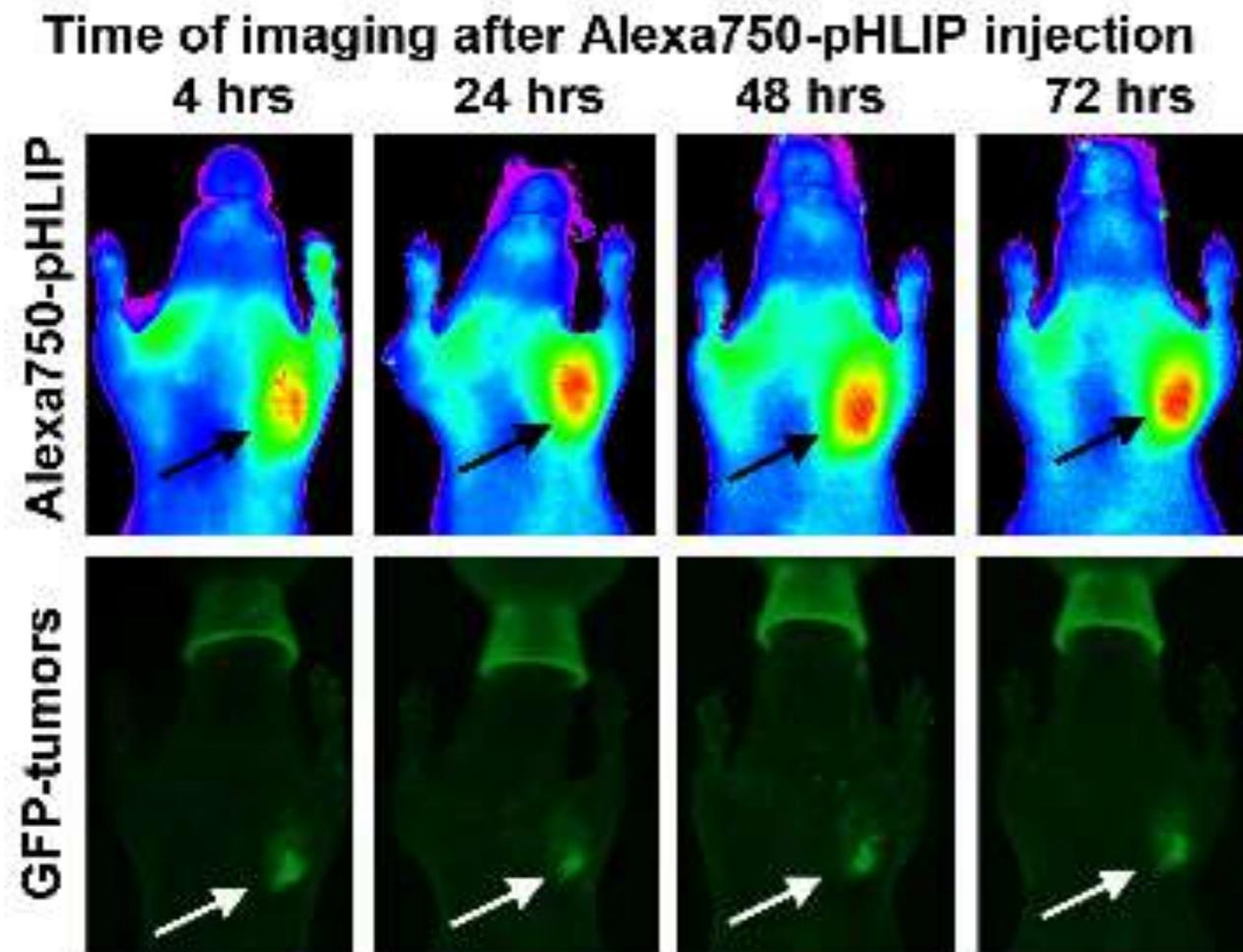
Drug delivery



Imaging tumors in vivo: pHILIP finds and accumulates in tumors

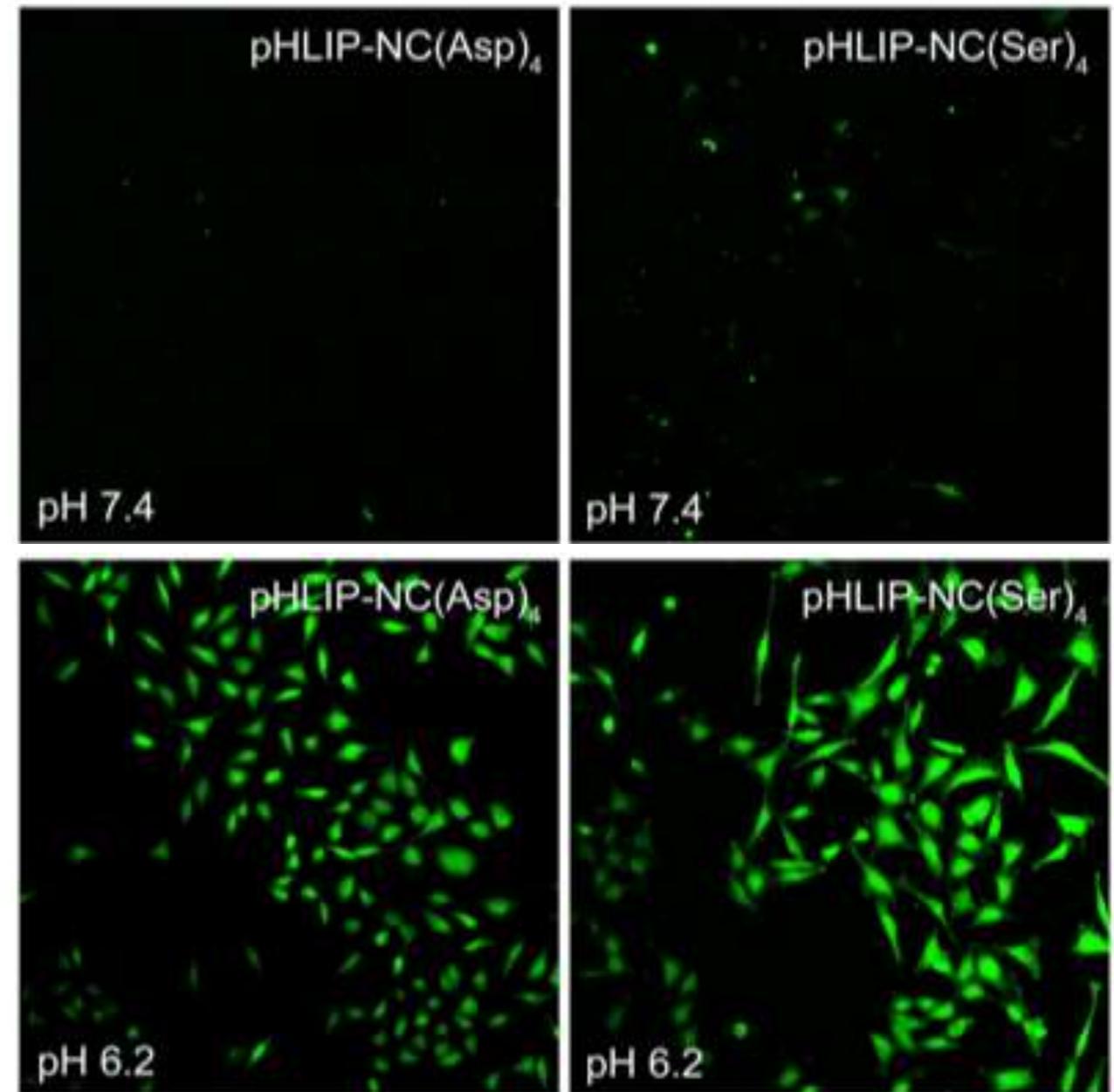
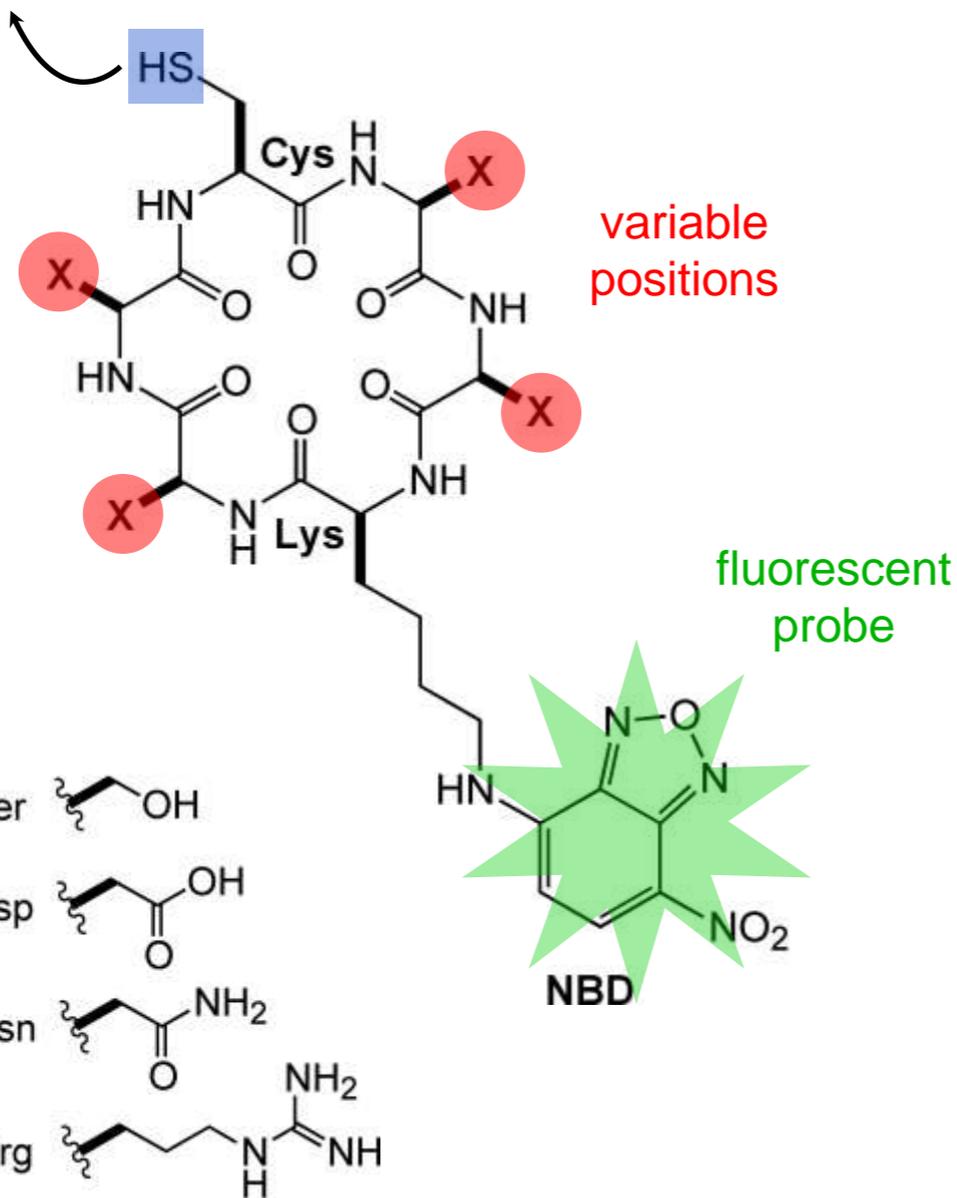
Nude mouse with cancer cells expressing the Green Fluorescent Protein (GFP)

pHLIP can help detecting sub-millimeter tumors



Delivery of cell-impermeable molecules into cells

Conjugation to
pHLIP



Conclusions

- Some of the drug delivery methods are in successful clinical use.
- Many are still in pre-clinical or clinical trials.
- Use of these methods (likely in combination with other approaches (surgery, radiation, chemotherapy) is making patient outcomes more positive.
- But, there is still a lot more work to do!