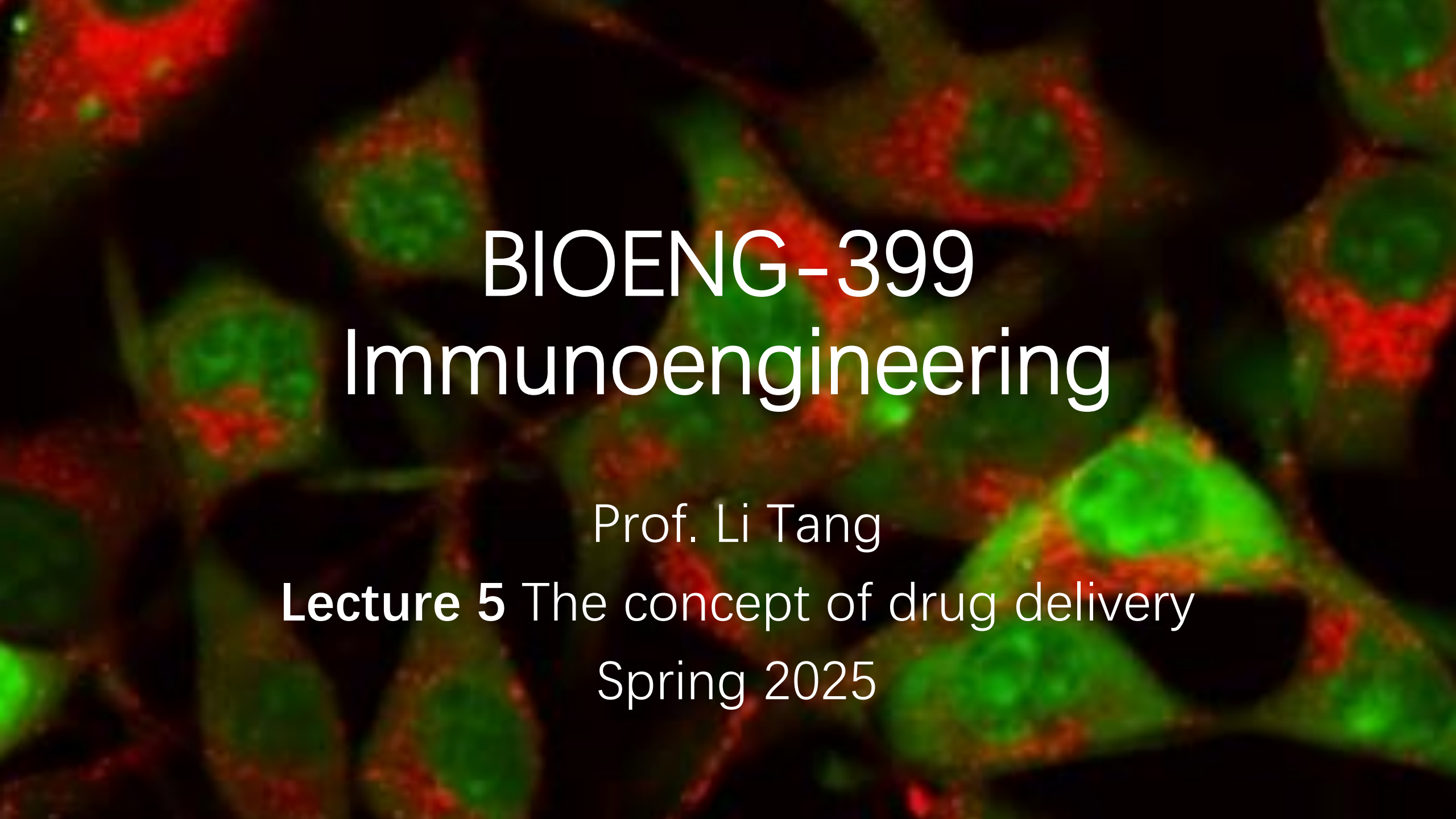


BIOENG-399 Immunoengineering

Prof. Li Tang

Lecture 5 The concept of drug delivery

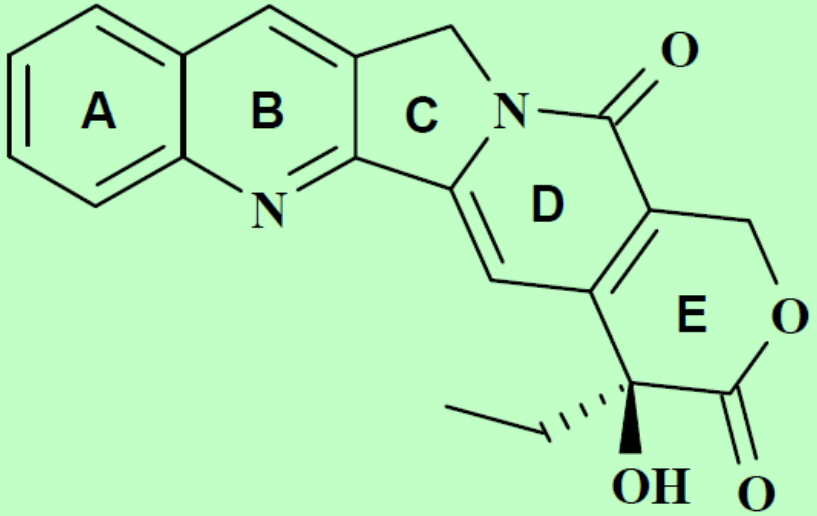
Spring 2025

Motivations for developing drug delivery system

- Poor solubility or stability of many drugs
- Rapid clearance
- Susceptibility to enzyme degradation
- Poor availability to tissues of interest
- Systemic toxicity and side effect

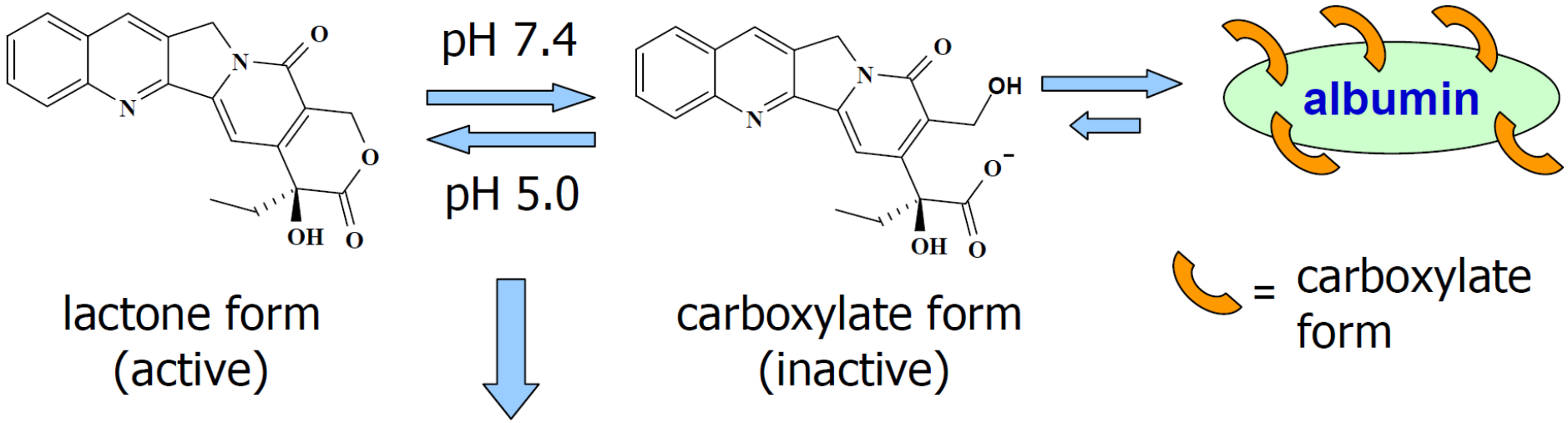
An example

Camptothecin (CPT) is a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase I (topo I).
It was first discovered in 1960, tested in human in 1970 failed to give any efficacy in vivo.



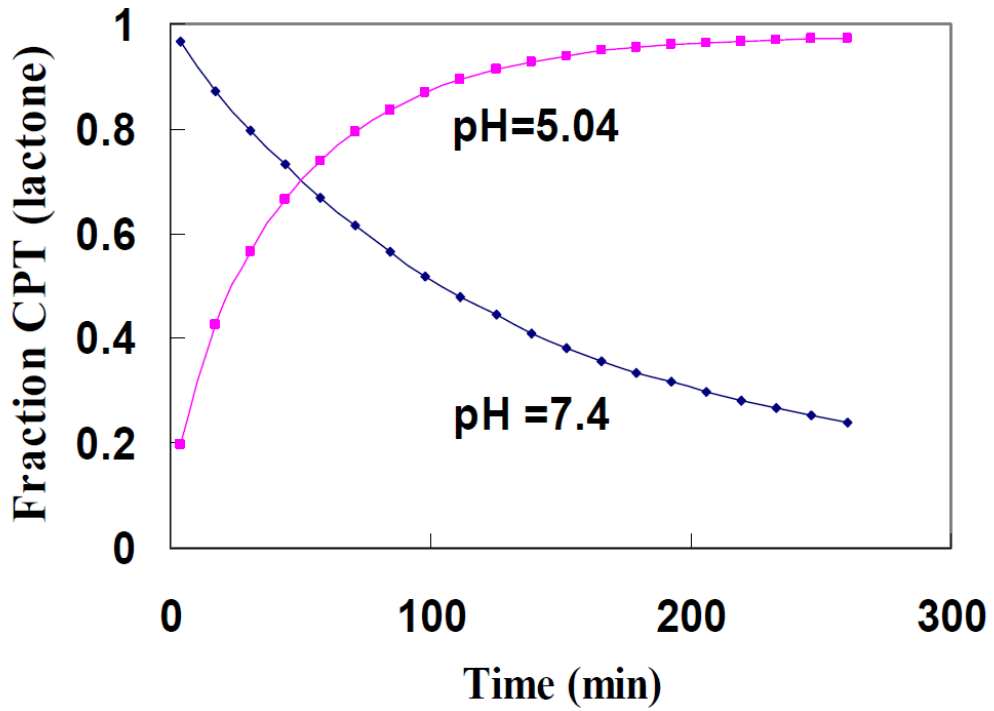
CPT

- Broad spectrum, potent antitumor activity
- Low solubility
- High toxicity



lactone form (active)

carboxylate form (inactive)



- CPT deactivated quickly in physiological condition

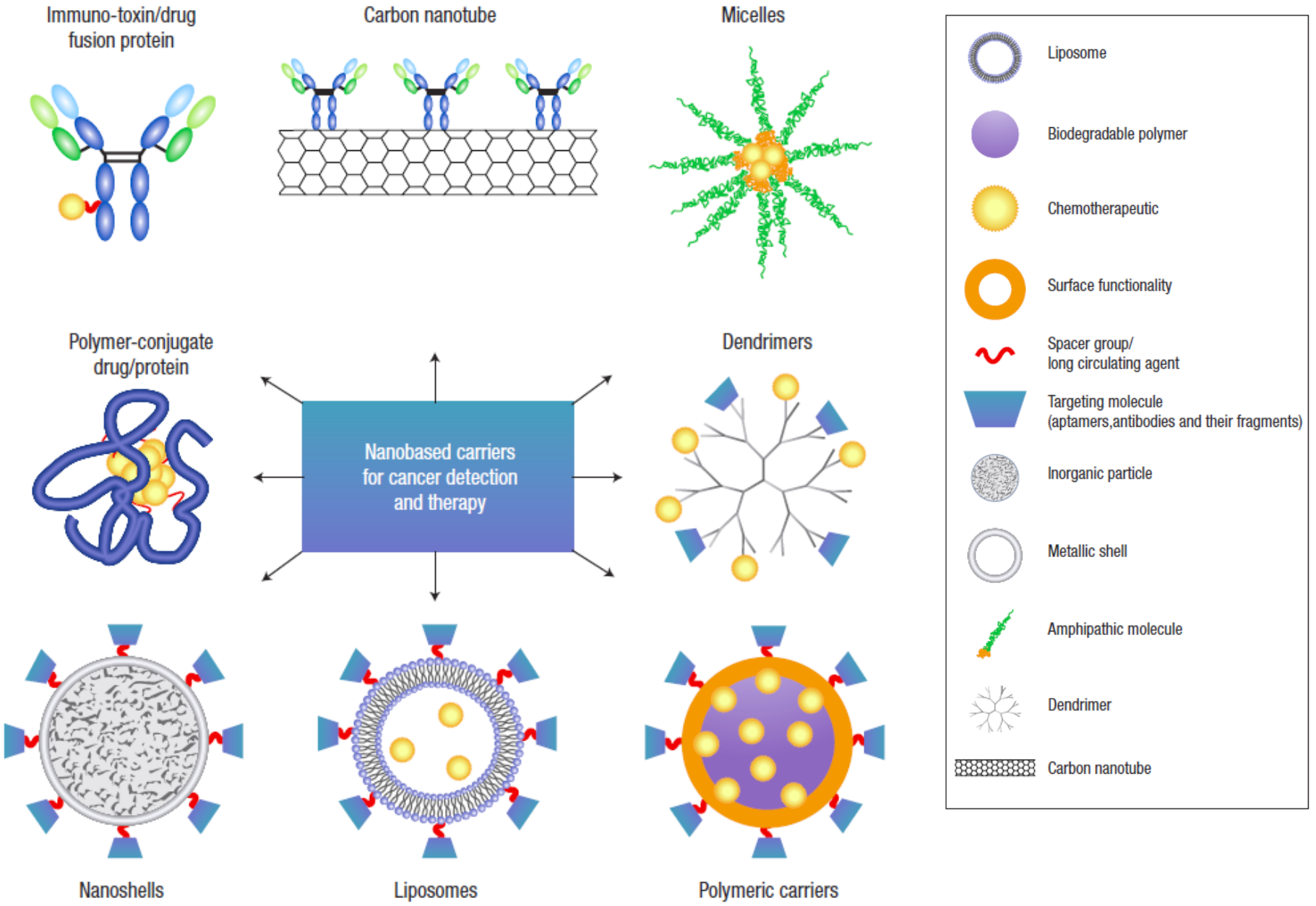
Overview of drug delivery

Drug delivery system (DDS)

Problem	Effect of DDS
Poor solubility	DDS such as lipid micelles or liposomes provide both hydrophilic and hydrophobic environments, enhancing drug solubility.
Tissue damage on extravasation	Regulated drug release from the DDS can reduce or eliminate tissue damage on accidental extravasation.
Rapid breakdown of the drug in vivo	DDS protects the drug from premature degradation and functions as a sustained release system. Lower doses of drug are required.
Unfavorable pharmacokinetics	DDS can substantially alter the PK of the drug and reduce clearance. Rapid renal clearance of small molecules is avoided.
Poor biodistribution	The particulate nature of DDS lowers the volume of distribution and helps to reduce side effects in sensitive, nontarget tissues.
Lack of selectivity for target tissues	DDS can increase drug concentrations in diseased tissues such as tumors by the EPR effect. Ligand-mediated targeting of the DDS can further improve drug specificity.

Allen and Cullis, *Science* **303**, 1818 (2004)

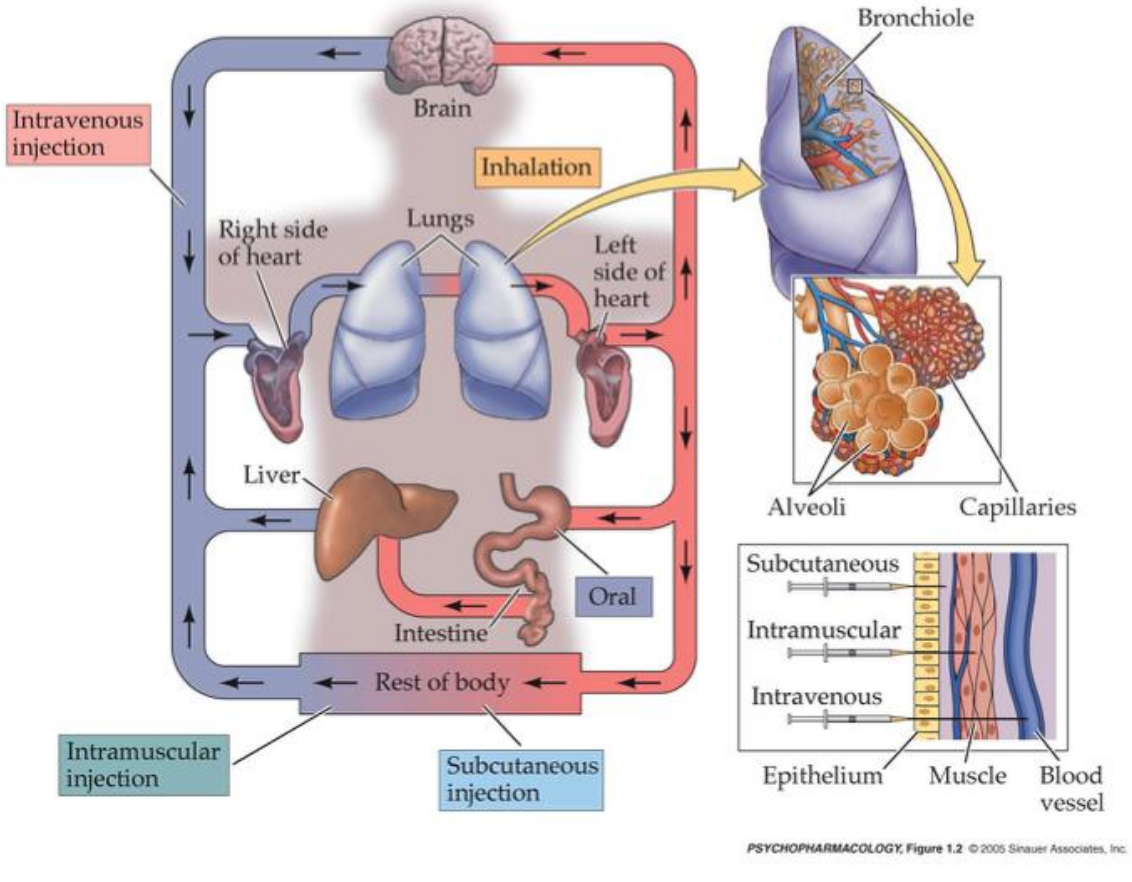
Overview of drug delivery carriers



Nat Nanotech, **2007**, 2, 751

Objectives of molecular and particulate drug/diagnostic/imaging agent carriers

- To alter pharmacokinetics
- To alter biodistribution
- To provide drug reservoirs (controlled release)



The ultimate goal of drug delivery

To deliver drug

INTACT

In the

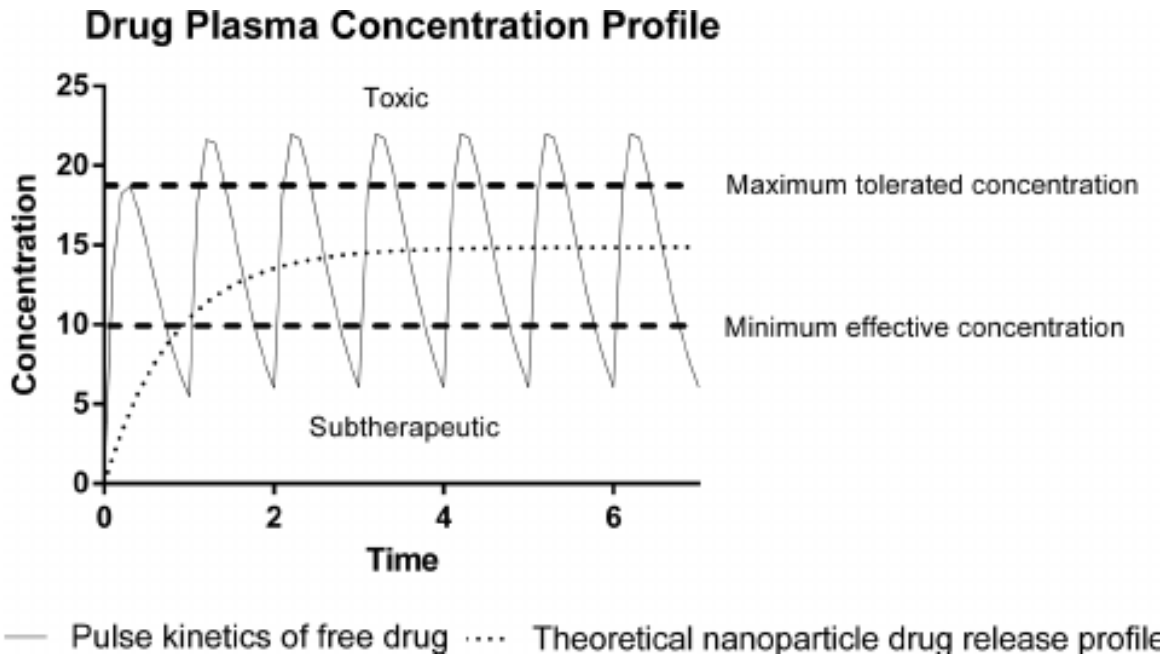
RIGHT AMOUNT

At the

RIGHT PLACE

And at the

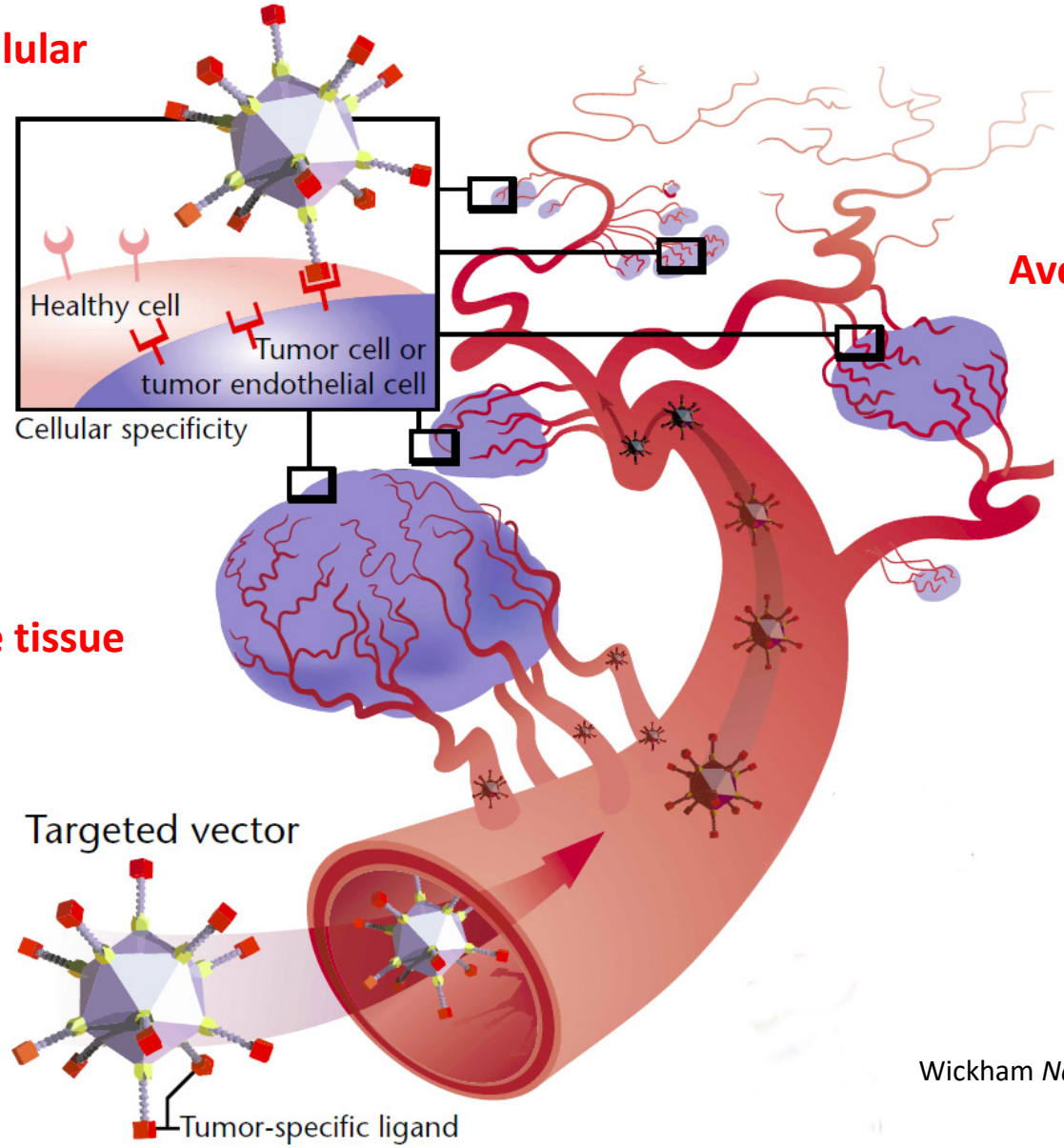
RIGHT TIME



https://www.researchgate.net/figure/271832084_fig3_Figure-3-Nanoparticle-pharmacokinetics-Drug-plasma-concentrations-associated-after

The concept of systemic delivery

Overcoming intracellular barriers



Avoiding clearance

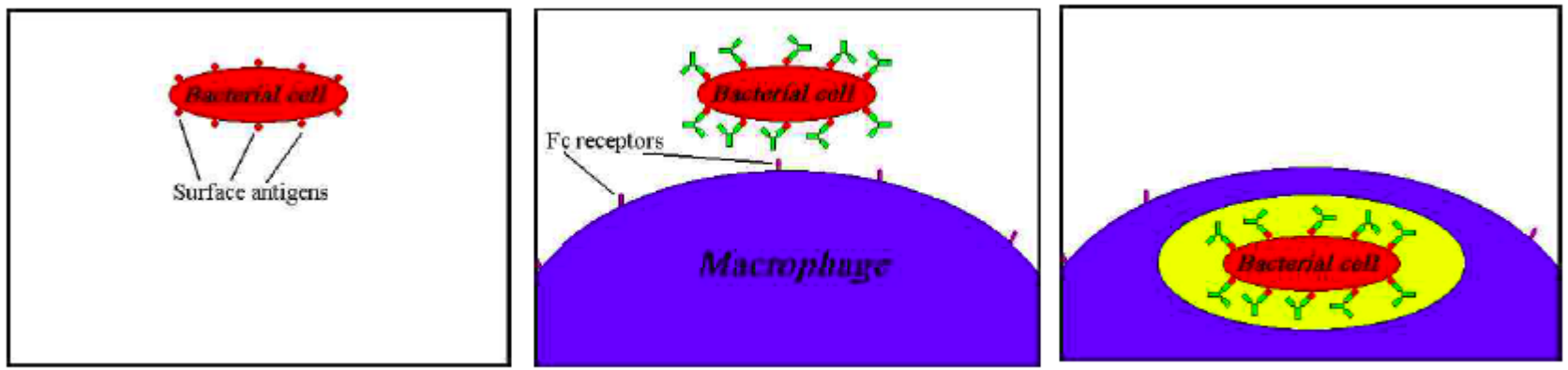
Targeting disease tissue

Injection to the bloodstream

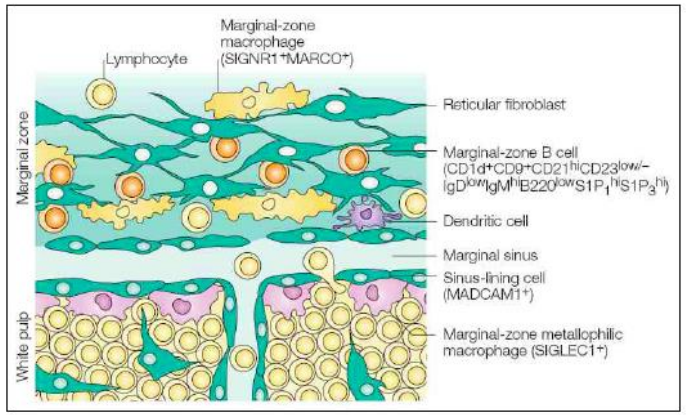
Wickham *Nat. Med*. **9** 135 (2003)

Clearance of nanoparticles

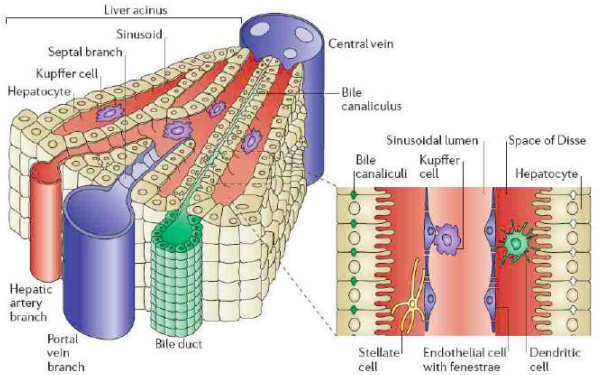
Carriers must avoid immune-mediated clearance



http://medtech.cls.msu.edu/ISL/immunology/opsonize.htm



Mebius and Kraal Nature Reviews Immunology 5, 606-616 (August 2005)



Adams and Ecksteen, Nat. rev. Immunol 6 244-251 (2006)

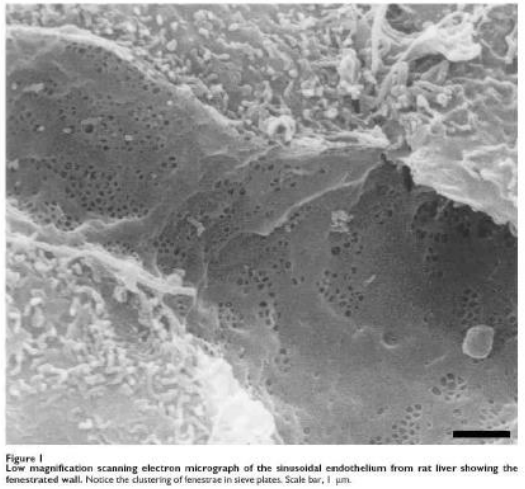


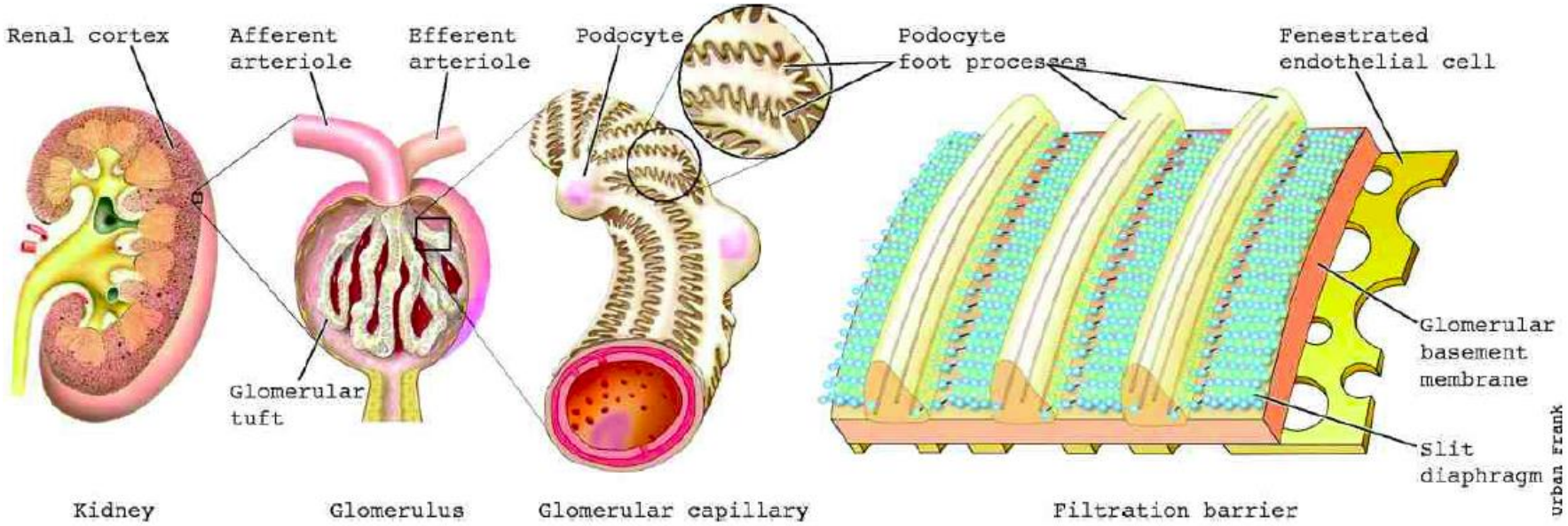
Figure 1
Low magnification scanning electron micrograph of the sinusoidal endothelium from rat liver showing the fenestrated wall. Notice the clustering of fenestrae in sieve plates. Scale bar, 1 μm.

(Braet and Wisse, *Comp. Hepatology* **1**, 1 (2002))

This slide is not required.

Clearance of nanoparticle carriers

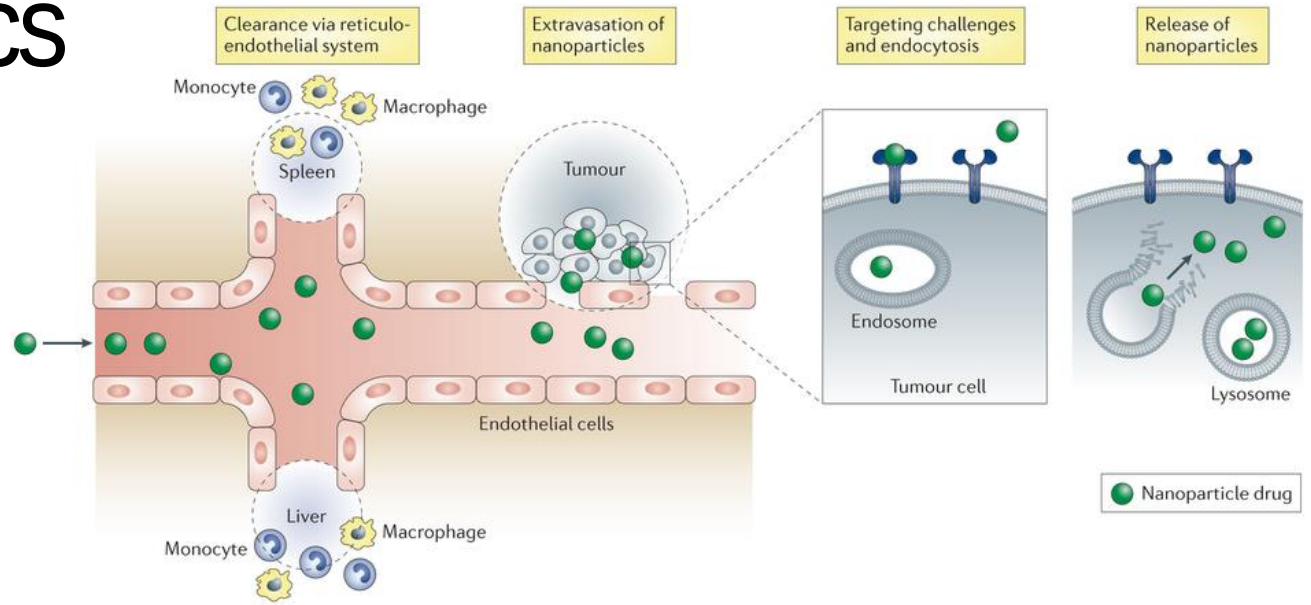
The kidneys are a size filter with small size cutoff (~10 nm):



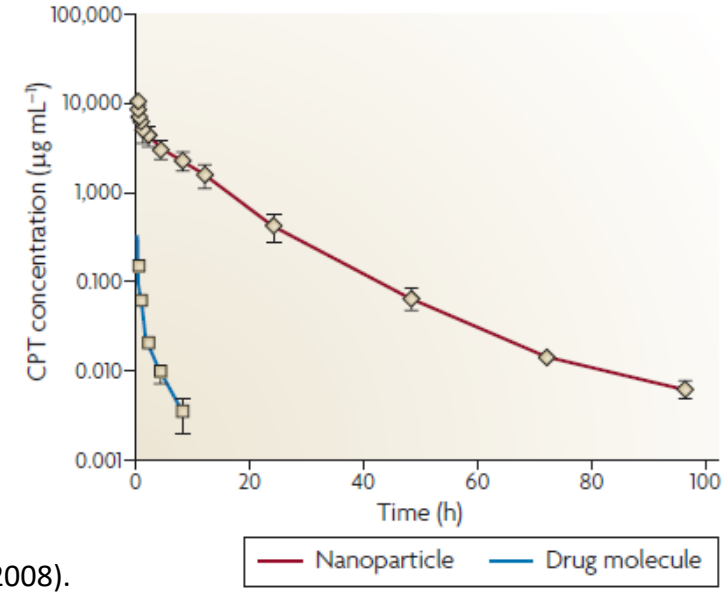
Tryggvason K , Wartiovaara J Physiology 2005;20:96-101

This slide is not required.

Clearance of nanoparticles and alteration of pharmacokinetics



Nature Reviews Drug Discovery 13, 655–672 (2014)s



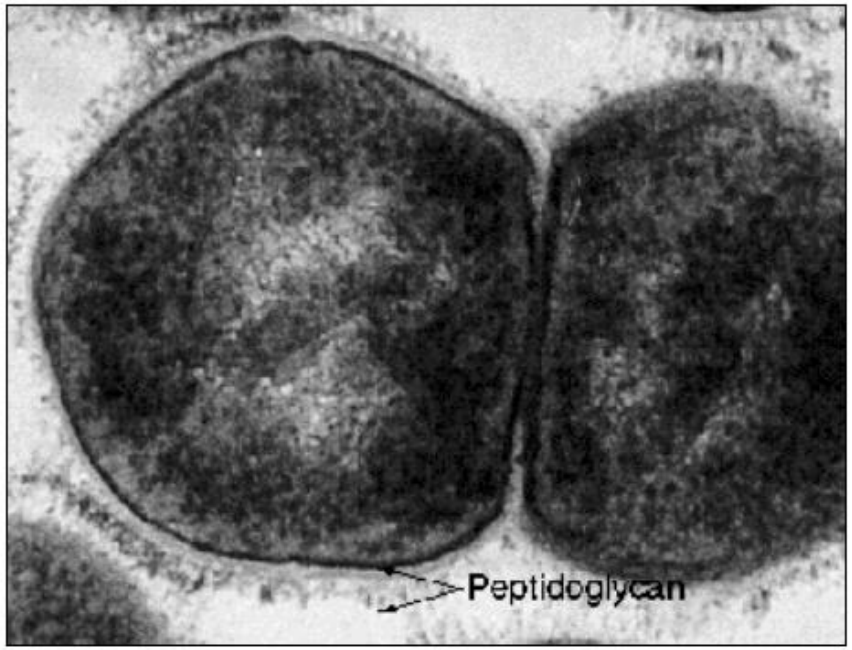
Nat. Rev. Drug Discov. **7**, 771-782 (2008).

Pharmacokinetics (PK) is the study of drug absorption, distribution, metabolism, and excretion (what the body does to a drug)

Half-life: period of time required for the concentration or amount of drug in the body to be reduced by one-half

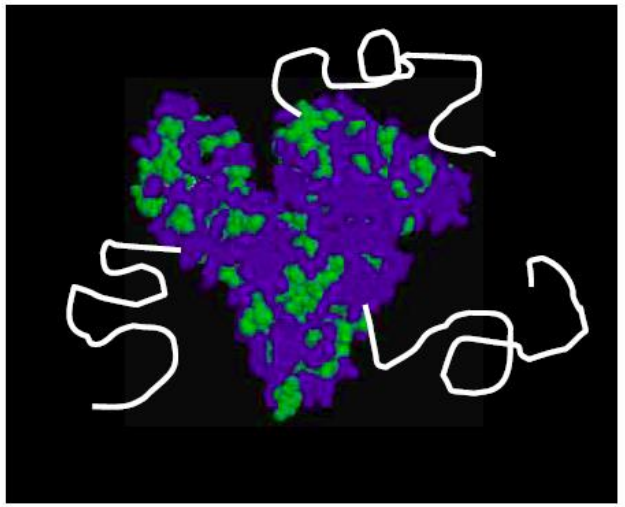
Alter pharmacokinetics with nanoparticle carriers

C. Van Oss (1978): showed that many bacteria which remain in circulation have a highly hydrophilic, hydrated surface layer of protein, polysaccharide, and glycoprotein



Annu Rev. Microbiol **32**, 19 (1978)

F.F. Davis (1977): showed showed that poly(ethylene glycol) conjugated to a protein is non-immunogenic and greatly increased protein half-lives *in vivo*



J. Biol. Chem. **252**, 3578 (1977)

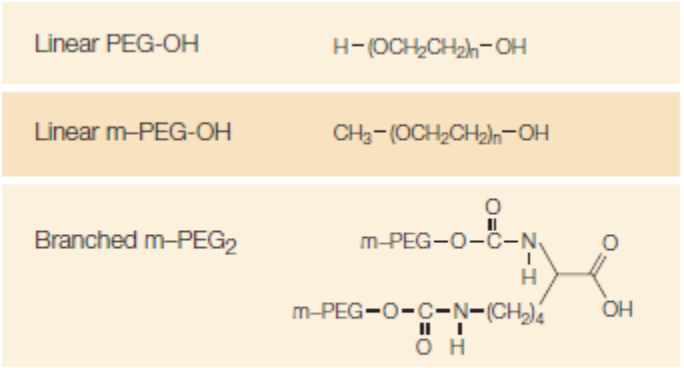


Figure 1 | **Structural formulae of polyethylene glycol (PEG) molecules.** m–PEG, monomethoxy–PEG.

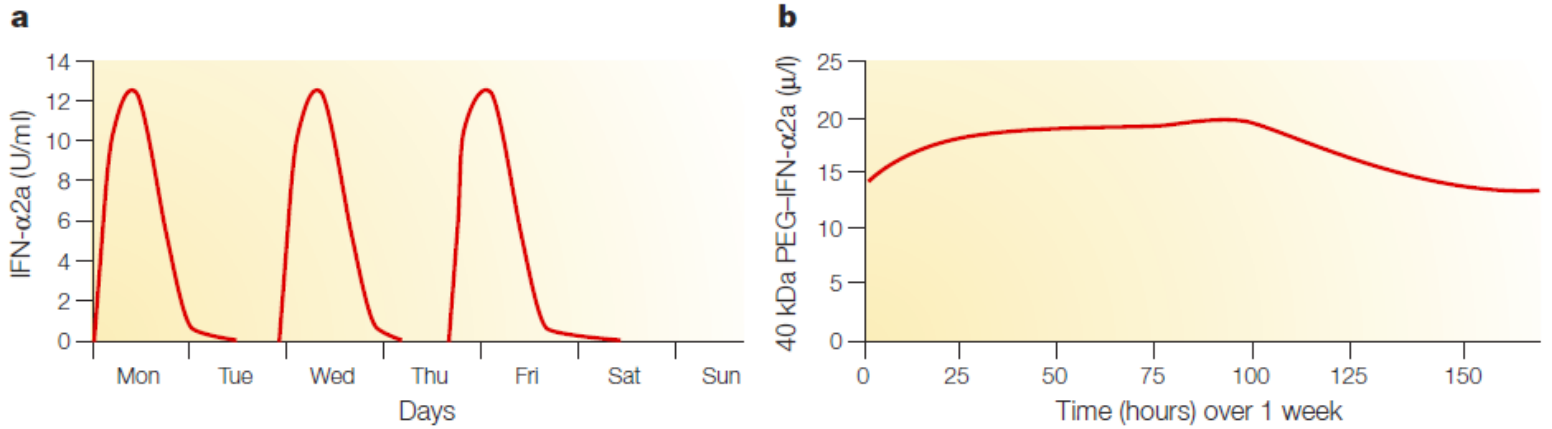
PEG: Polyethylene glycol
PEGylation: conjugate or modify with PEG

PEGylation of protein drugs

Table 1 | **Influence of pegylation on pharmacokinetics and pharmacodynamics***

Pharmacokinetic effect	Pharmacodynamic effect
Interferon-α2a	
Sustained absorption	*In vivo* antiviral activity increased 12- to 135-times
Increased half-life (from 3–8 h to 65 h)	Antitumour activity increased 18-fold
Decreased volume of distribution (from 31–73 l to 8–12 l)	Improved sustained response to chronic hepatitis C
Decreased systemic clearance (from 6.6–29.2 to 0.06–0.10 l/h)	
Interleukin-6	
Increased half-life (from 2.1–206 min)	Thrombopoietic potency increased 500-times
Tumour necrosis factor	
Increased half-life (from 3 to 45–136 min)	Antitumour potency increased 4-to 100-times

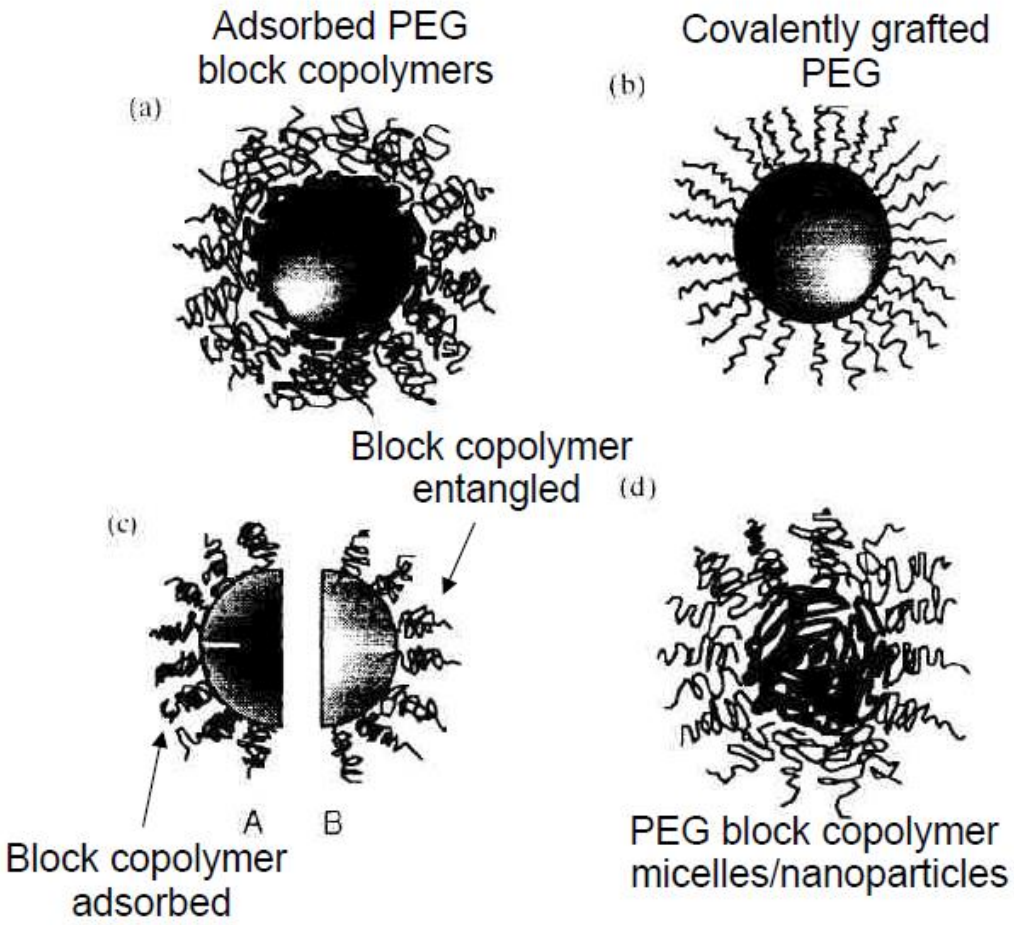
*Influence of pegylation on pharmacokinetics and pharmacodynamics of some therapeutic proteins, compared with corresponding native proteins (adapted from REF. 18).



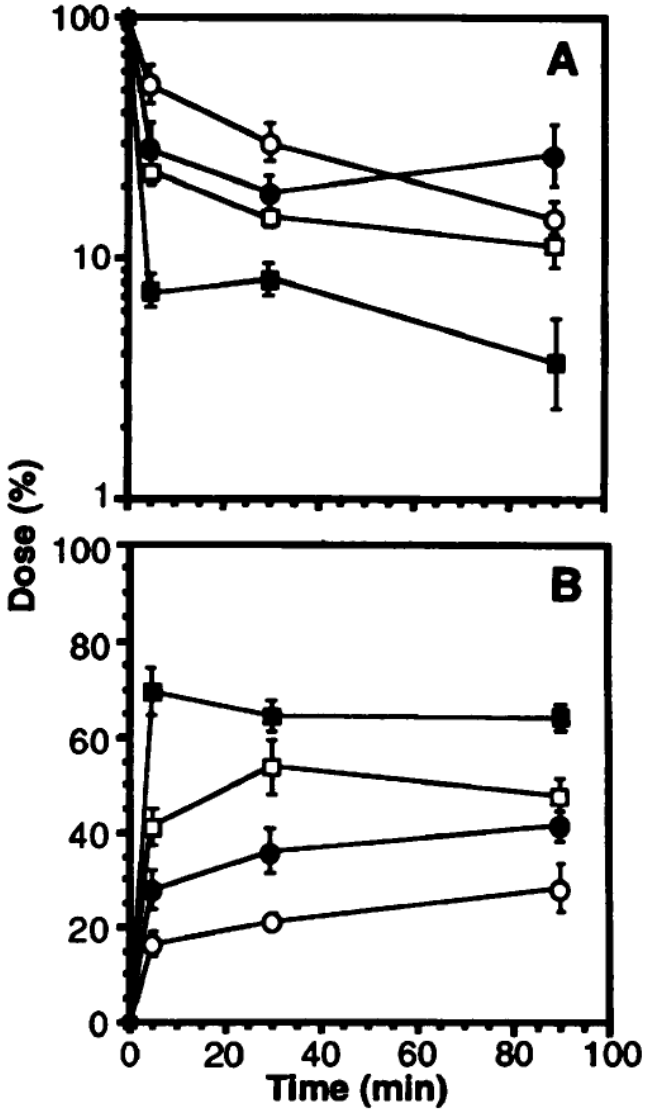
Nat. Rev. Drug Discov. **2**, 214-221 (2003).

PEGylation of nanoparticles

This slide is not required.



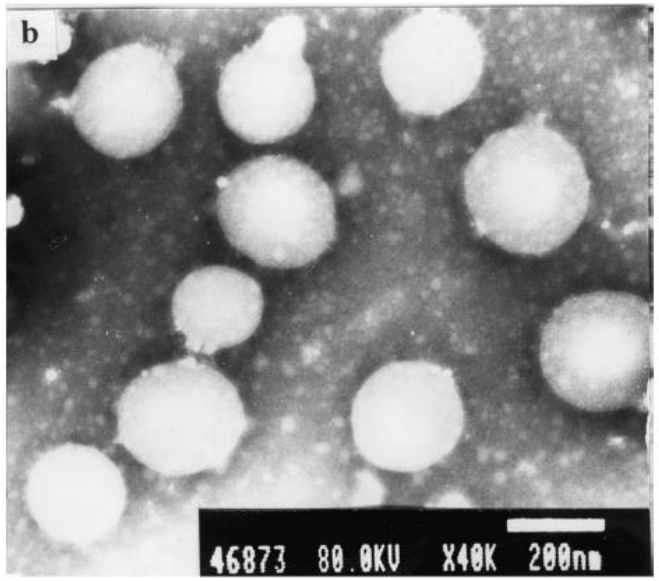
Stolnik et al. 1995



Greg, Langer et al. Science 263 1600-1603 (1994)

PEGylation of nanoparticles

This slide is not required.



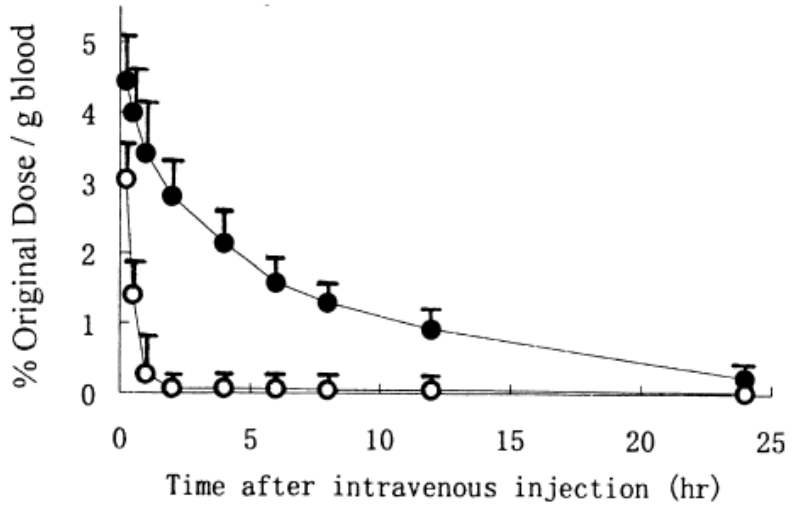
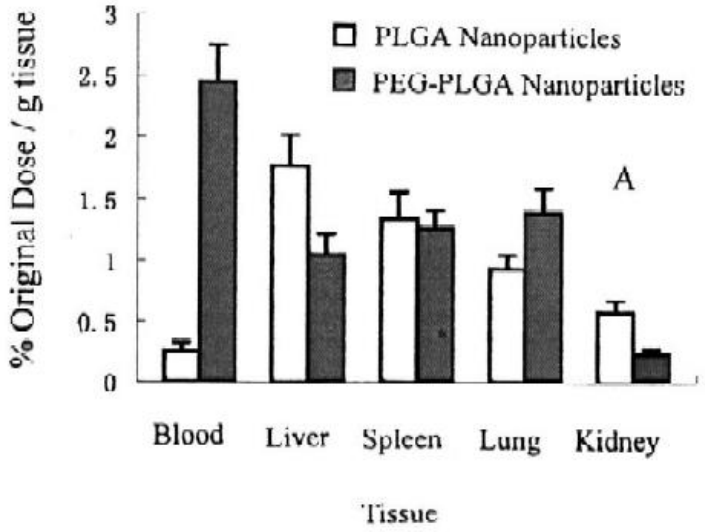


Fig. 7. Blood clearance curves of [^{125}I]BSA in PLGA (○) and PEG–PLGA (●) nanoparticles.



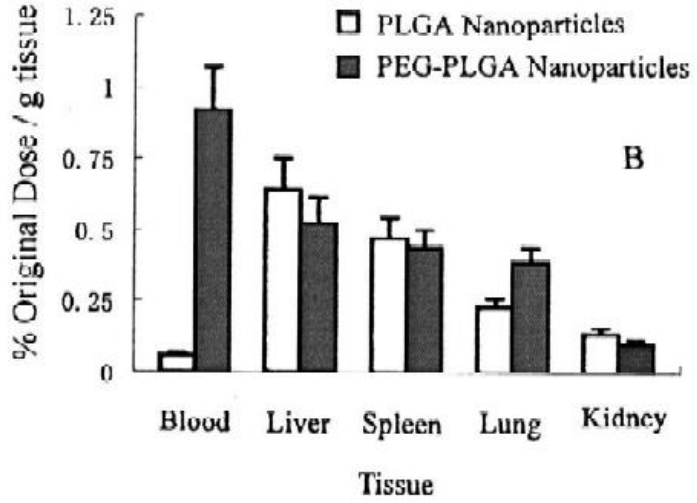


Fig. 8. Tissue distribution of [^{125}I]BSA at 3 h (A) and 12 h (B) in PLGA and PEG–PLGA nanoparticles.

Journal of Controlled Release 71 (2001) 203–211

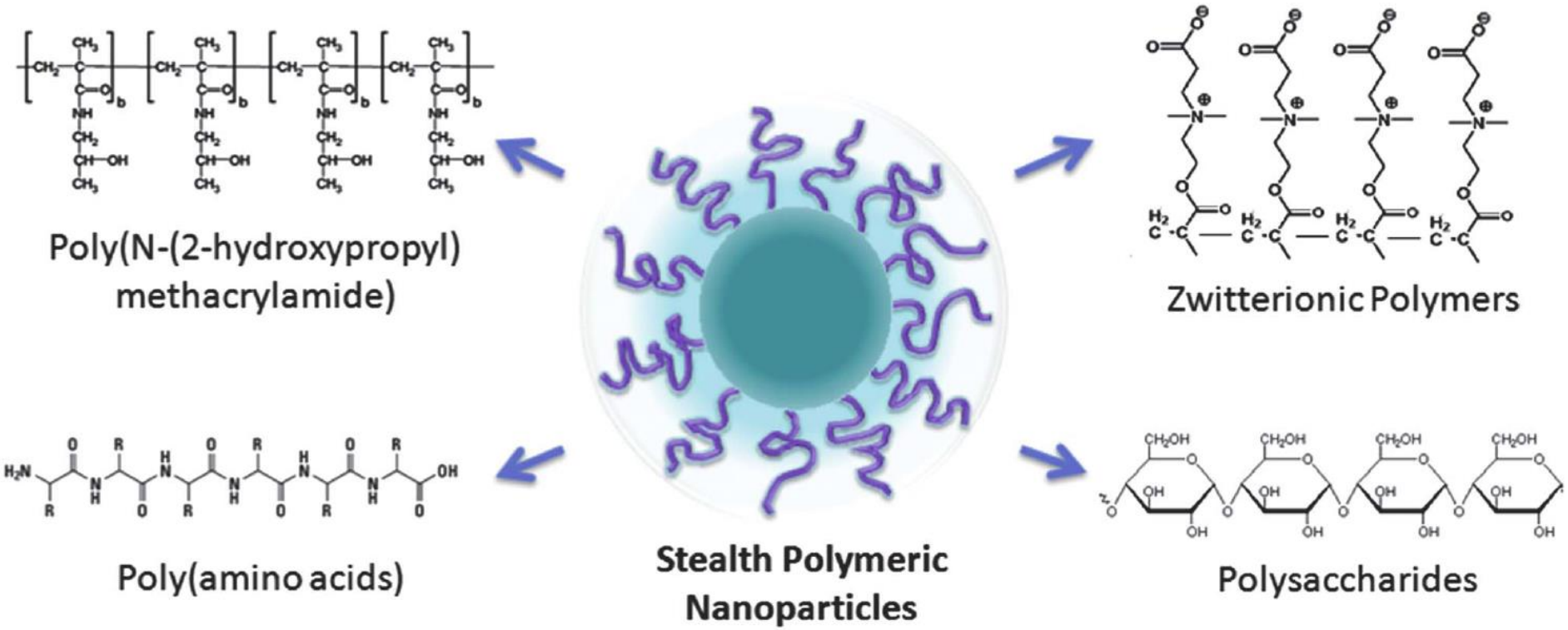
Table 2 | **Comparison of pharmacokinetics (human) of small-molecule drugs with nanoparticle therapeutics**

Name	Formulation	Diameter (nm)	$t_{½}$ (h)	Clearance (ml/min•kg)	Comments	Refs
Doxorubicin (DOX)	0.9% NaCl	NA	0.8	14.4	Small-molecule drug	24
SP1049C	Pluronic micelle + DOX	22–27	2.4	12.6	Micelle nanoparticle	24
NK911	PEG–Asp micelle + DOX	40	2.8	6.7	Micelle nanoparticle	24
Doxil	PEG–liposome + DOX	80–90	84.0	0.02	PEGylated liposome nanoparticle with long circulation	24
Taxol (paclitaxel)	Cremophor EL	NA	21.8 (20.5)	3.9 (9.2)	Small-molecule drug	24 (28)
Genexol-PM	PEG–PLA micelle + paclitaxel	20–50	11.0	4.8	Micelle nanoparticle	24
Abraxane	Albumin + paclitaxel	120*	21.6	6.5	Albumin nanoparticle before injection; status *in vivo* unknown	28
XYOTAX	PG + paclitaxel	Unknown	70–120	0.07–0.12	Polymer nanoparticle	23
Camptosar (prodrug of SN-38)	0.9% NaCl	NA	11.7	5.8	Small-molecule prodrug	95
LE-SN-38	Liposome + SN-38	Unknown	7–58	3.5–13.6	Liposome nanoparticle	97
Topotecan (camptothecin analogue)	0.9% NaCl	NA	3.0	13.5	Small-molecule drug	96
CT-2106	PG + camptothecin	Unknown	65–99	0.44	Polymer nanoparticle	98
IT-101	Cyclodextrin-containing polymer + camptothecin	30–40	38	0.03	Polymer nanoparticle with extended circulation times	66

*May dissolve upon exposure to blood. NA, not applicable; PEG, polyethylene glycol; PEG–PLA, block copolymer of PEG and poly(L-lactic acid); PG, polyglutamic acid; SN-38, 7-ethyl-10-hydroxycamptothecin.

Nat. Rev. Drug Discov. **7**, 771-782 (2008).

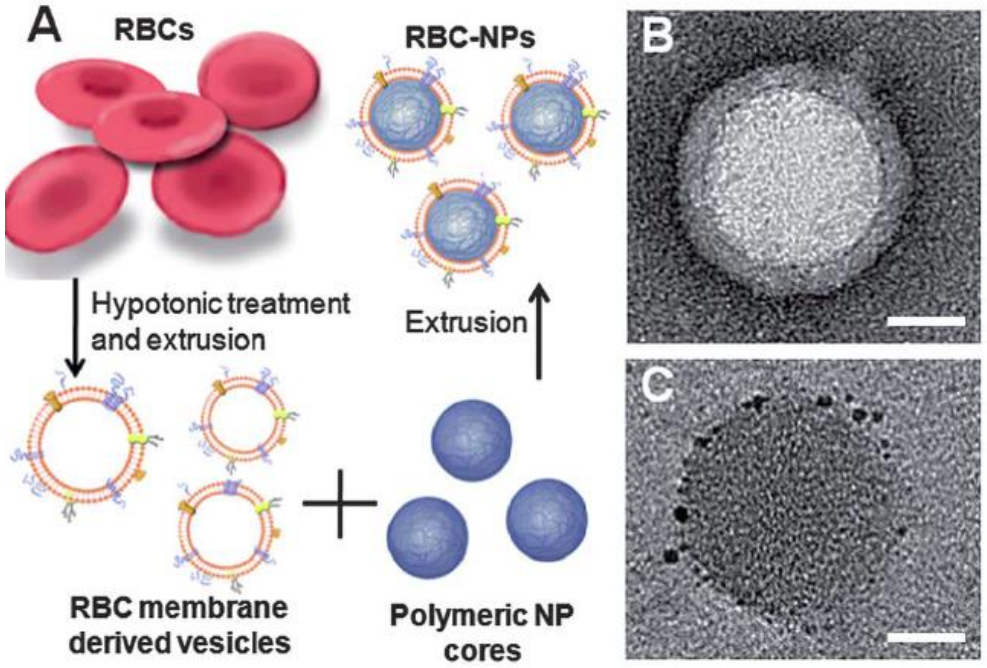
Other polymer to modify the nanoparticle surface

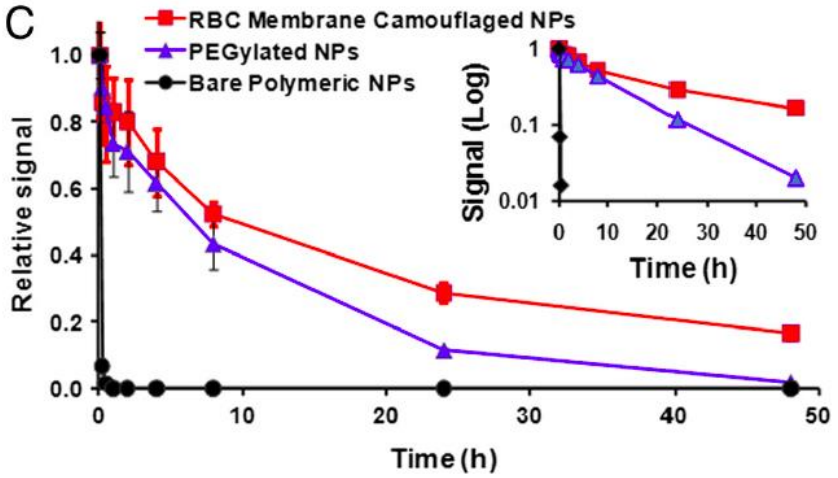


Nanoscale, 2014, 6, 65

Biomimetic nanoparticles

scale bar =20 nm





(B) TEM visualization of the RBC–NP shows unilamellar membrane coating over the polymeric core

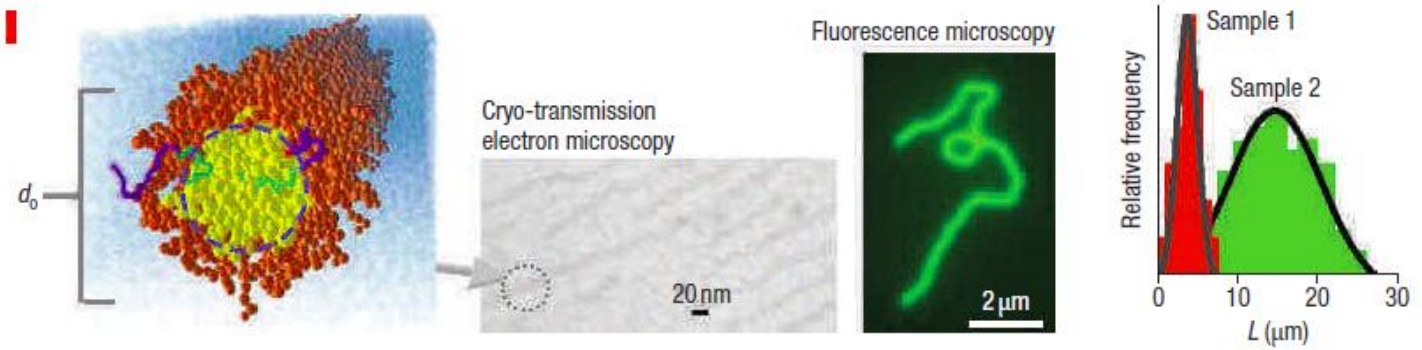
(C) Immunogold staining with anti-CD47 antibodies targeting the protein's extracellular domain

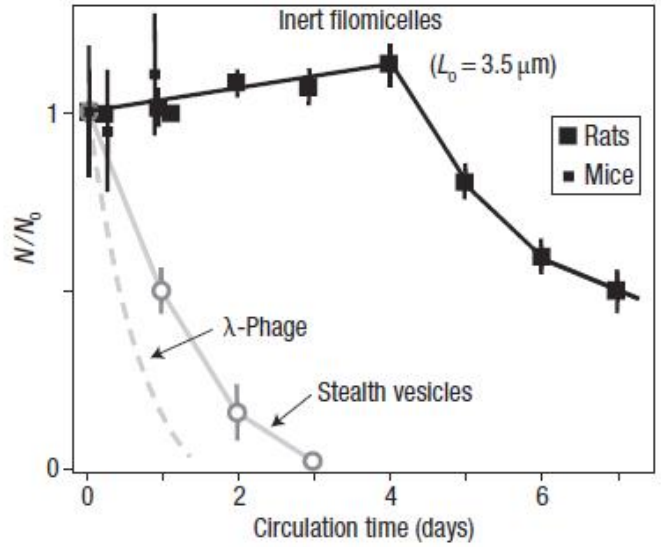
- Red-blood cell membrane coated polymer nanoparticles showed longer circulation half-life
- This is likely due to the CD47 receptor on the membrane, known as "don't eat me" signal

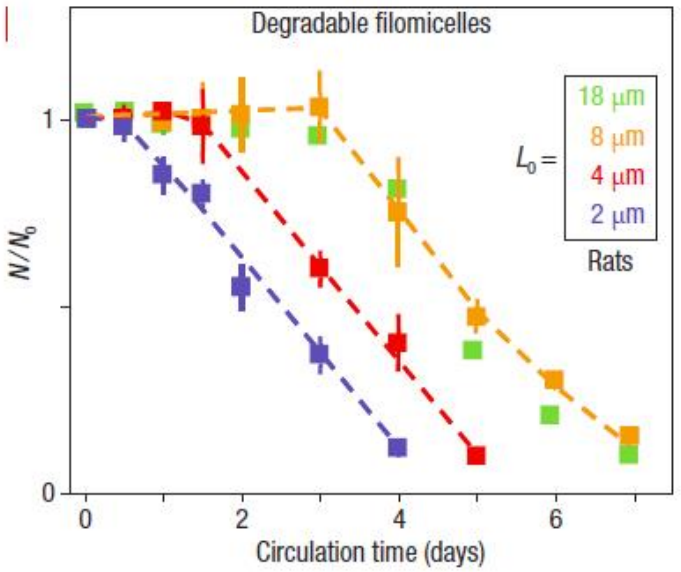
Nanoscale, 2014, 6, 65

Proceedings of the National Academy of Sciences **108**, 10980-10985 (2011).

Impact of particle geometry on circulation time





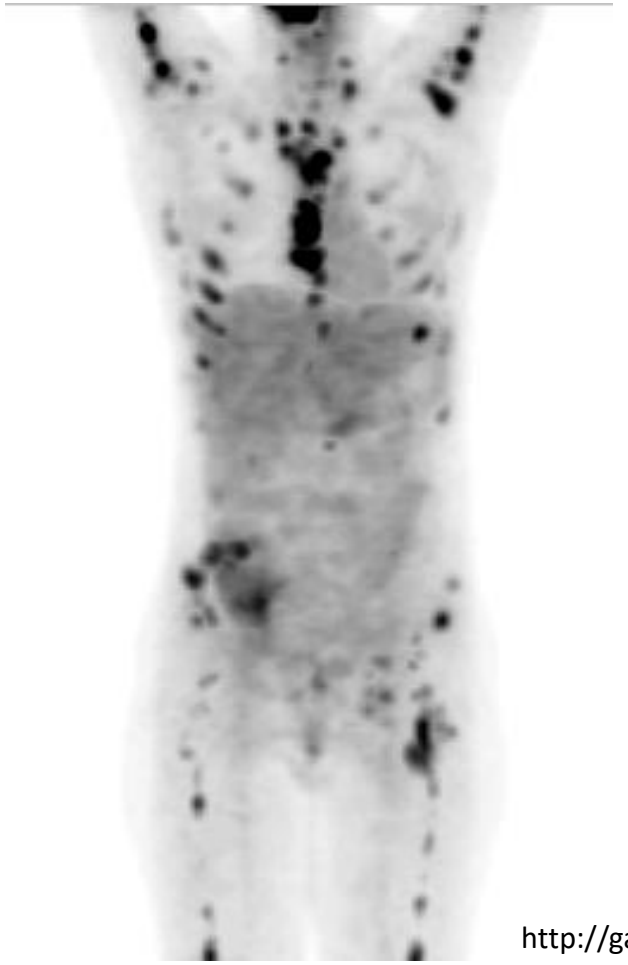


Nat. Nanotechnol. **2**, 249-255 (2007).

This slide is not required.

Another key challenge: targeted delivery of therapeutic or diagnostic agents to disseminated tissues

metastatic cancer



http://gamma.wustl.edu/

disseminated infections

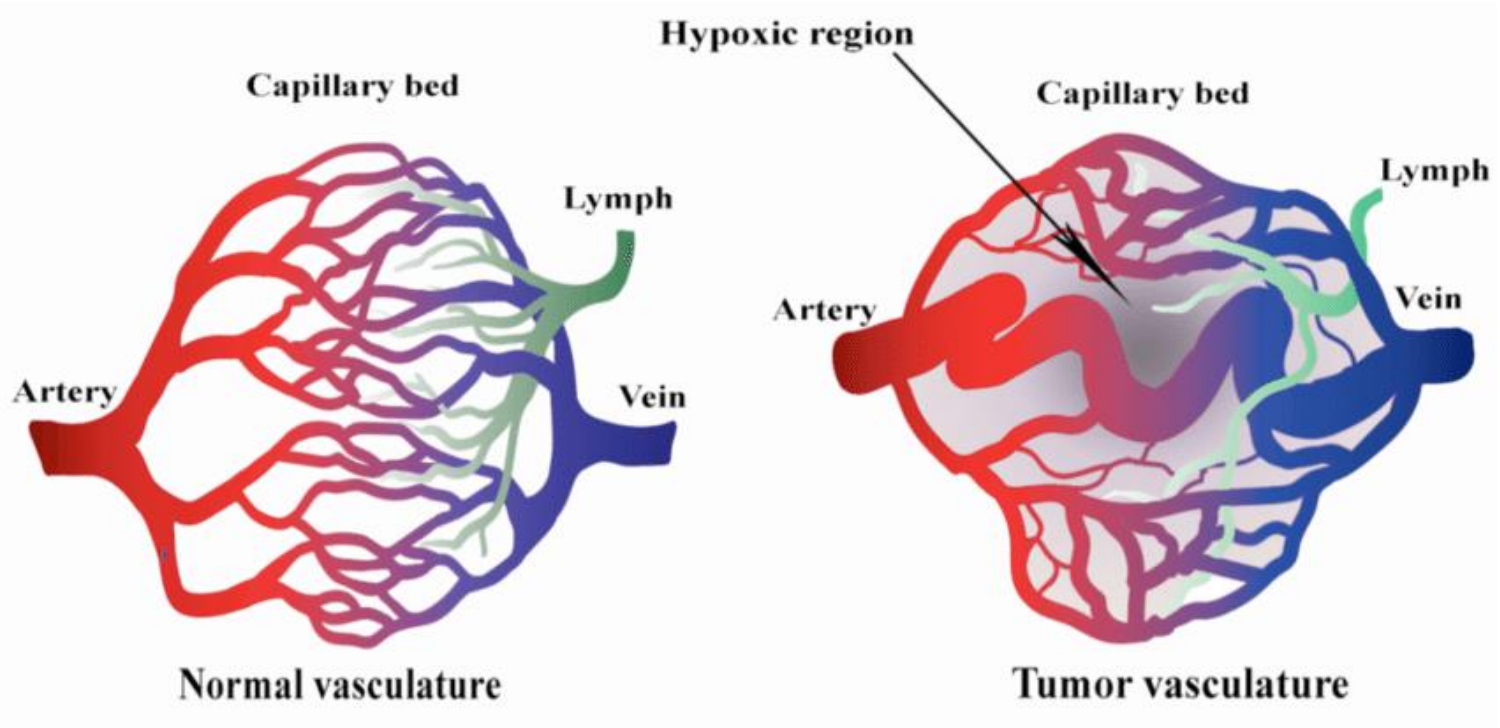
http://www.cram.com/

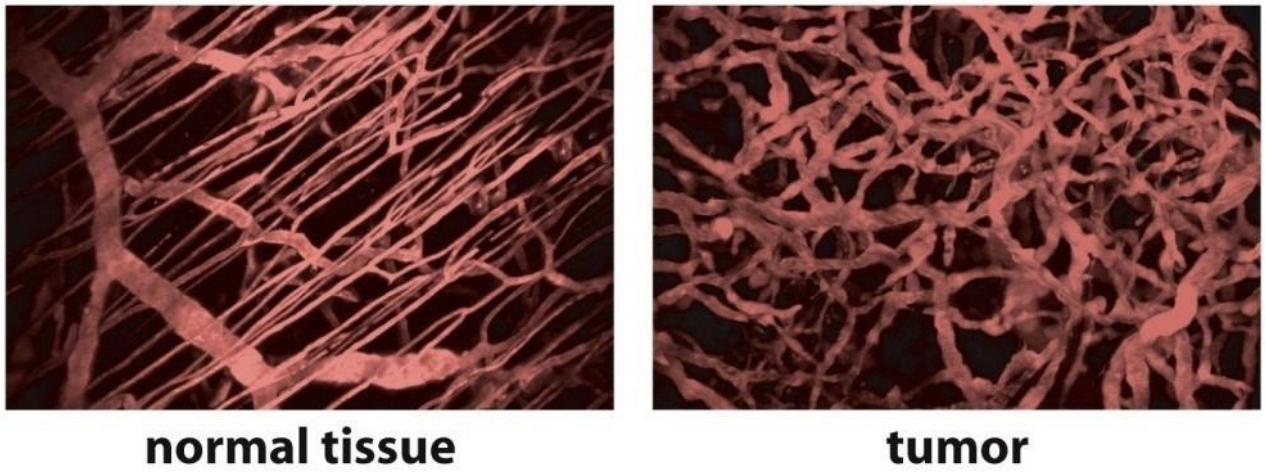
Strategies for systemic targeting

- Passive targeting
  - ➢ Enhanced permeation and retention (EPR) effect in tumors
- Active targeting
  - ➢ Antibody-based targeting

Enhanced permeation and retention effect in tumors

- Defective and chaotic vascular architecture
- impaired lymphatic drainage /recovery system

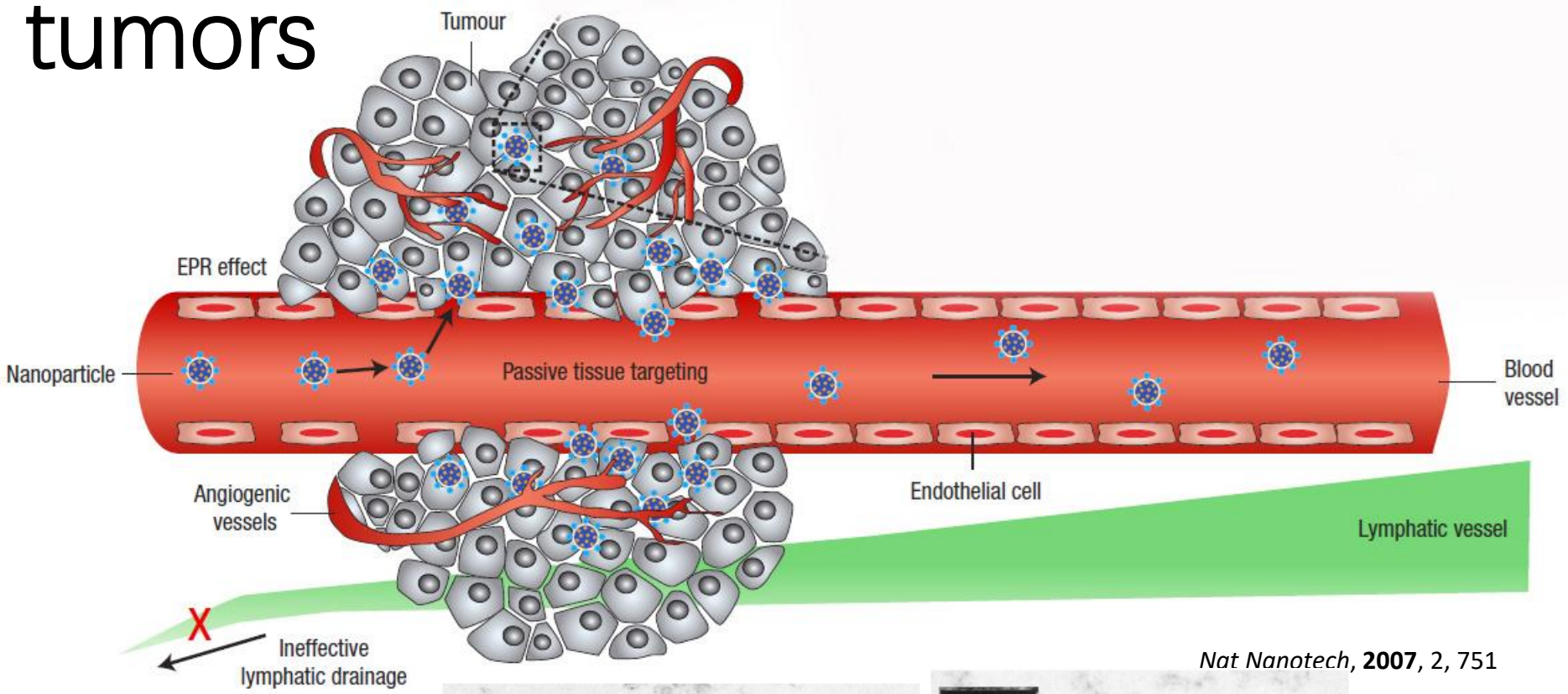




*Cell Death & Disease***volume 9**, Article number: 115 (2018)

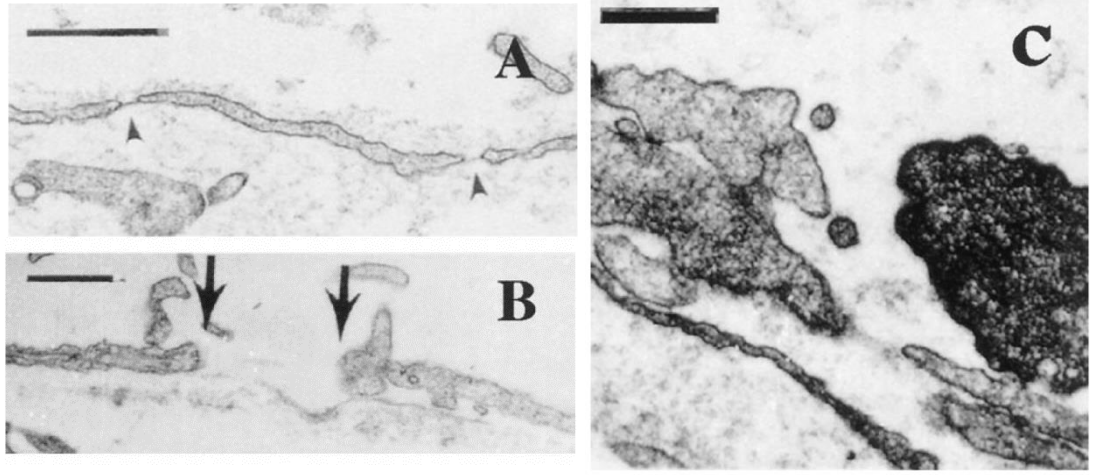
https://www.dovepress.com/a-review-of-the-development-of-tumor-vasculature-and-its-effects-on-th-peer-reviewed-article-HP

Enhanced permeation and retention effect in tumors



Nat Nanotech, **2007**, 2, 751

Pore size: 380-780 nm

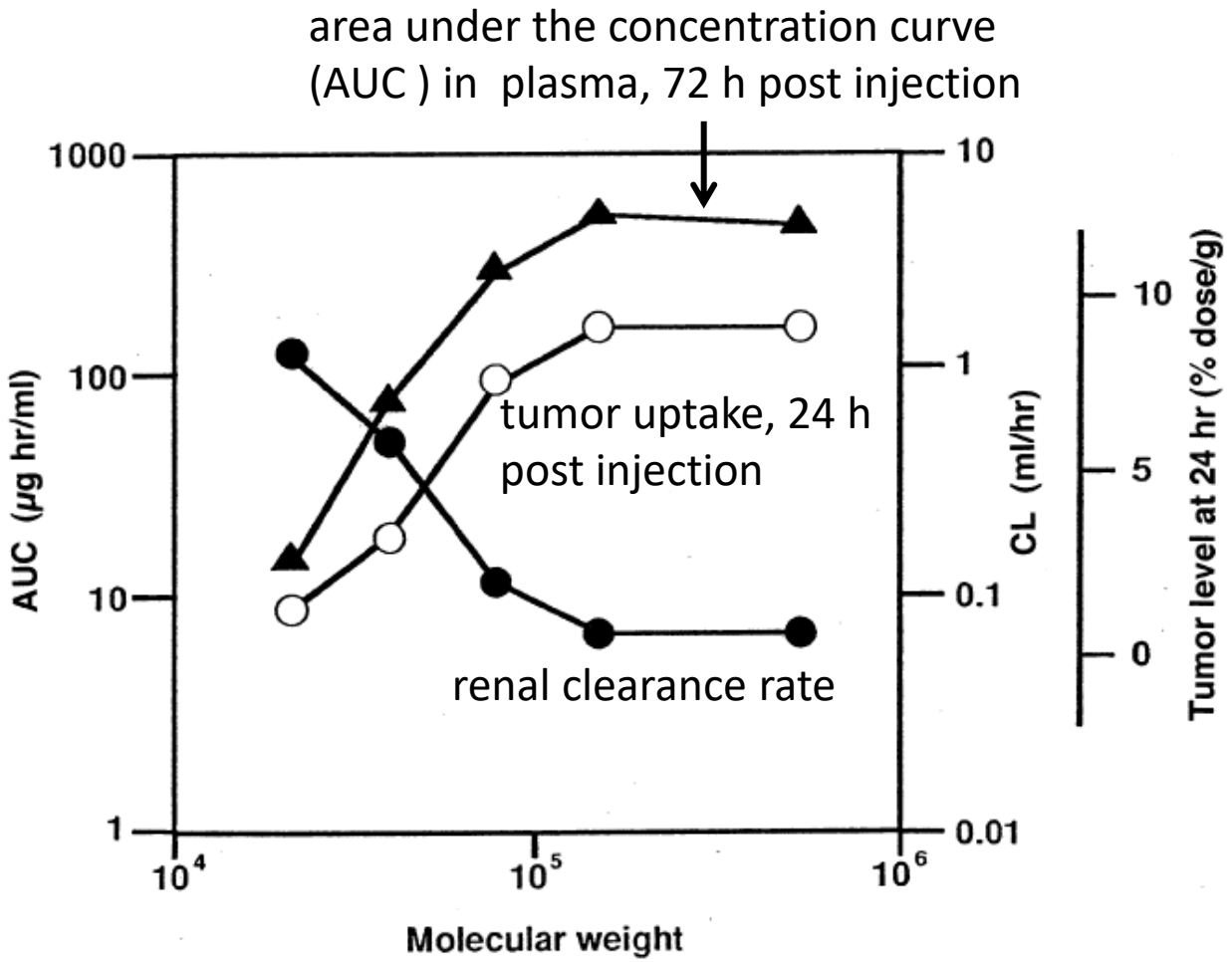


*PNAS, **1998**, 95, 4607*

EPR relies on specific pathophysiological characteristics of tumors vs. healthy tissues. In healthy tissues, low-molecular-weight drugs easily extravasate out of blood vessels, while nanomedicines are unable to do so, because of their size. Conversely, in tumors, the abnormally wide fenestrations in the blood vessels allow for the extravasation of materials with sizes up to several hundreds of nanometers. This, together with the absence of lymphatic drainage, leads to a relatively effective and selective accumulation of nanomedicines in tumors.

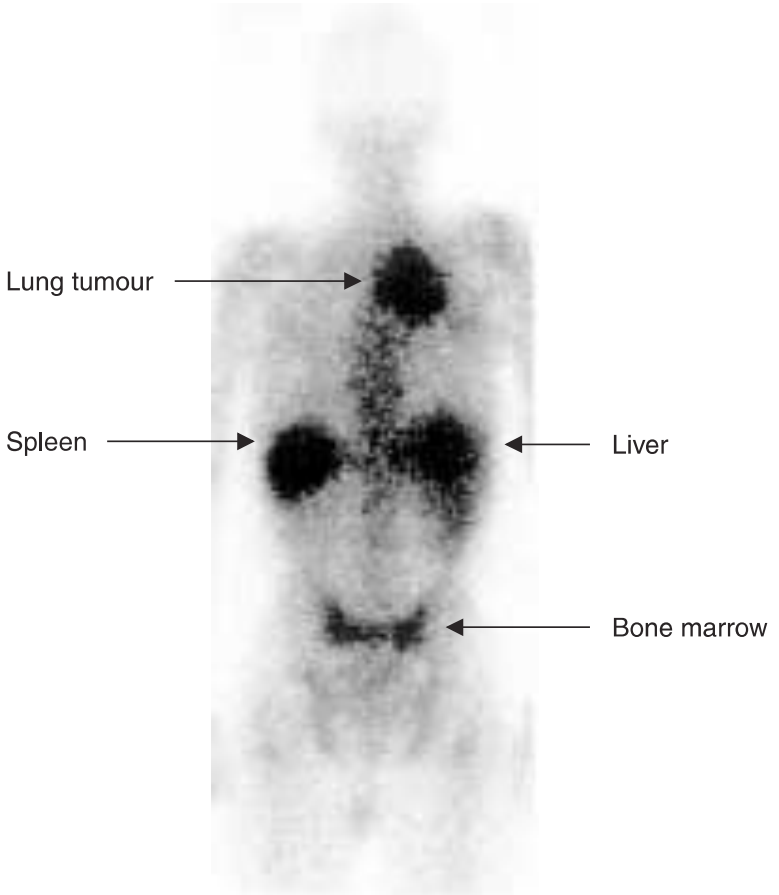
Enhanced permeation and retention effect in tumors

Maeda, et al, **1986**



J. Controlled Release **2000**, *65*, 271-284.

^{111}In-labeled PEGylated liposomes (Doxil) 48 hr after injection in lung cancer patient:

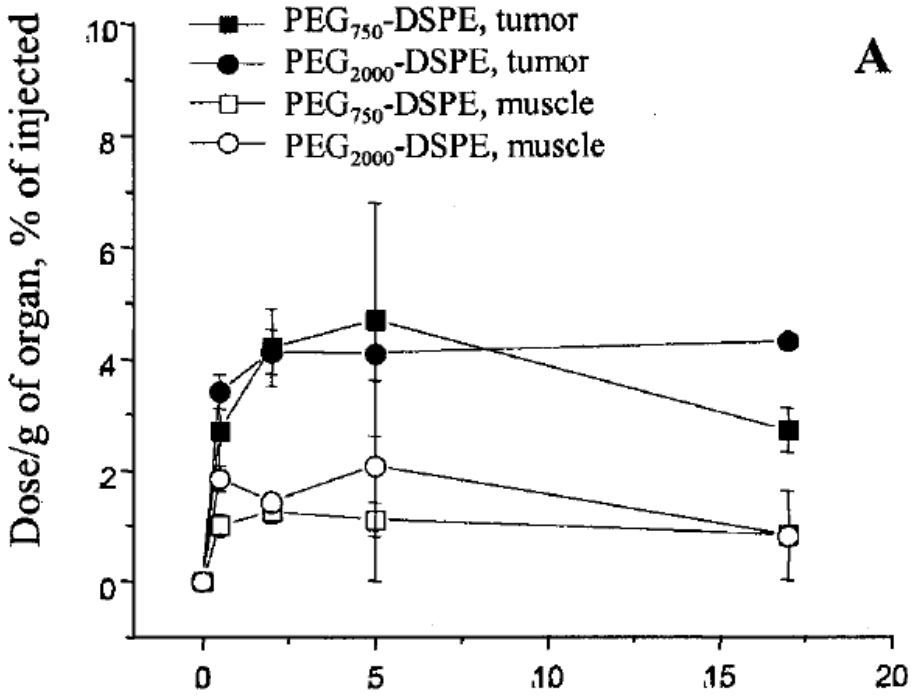


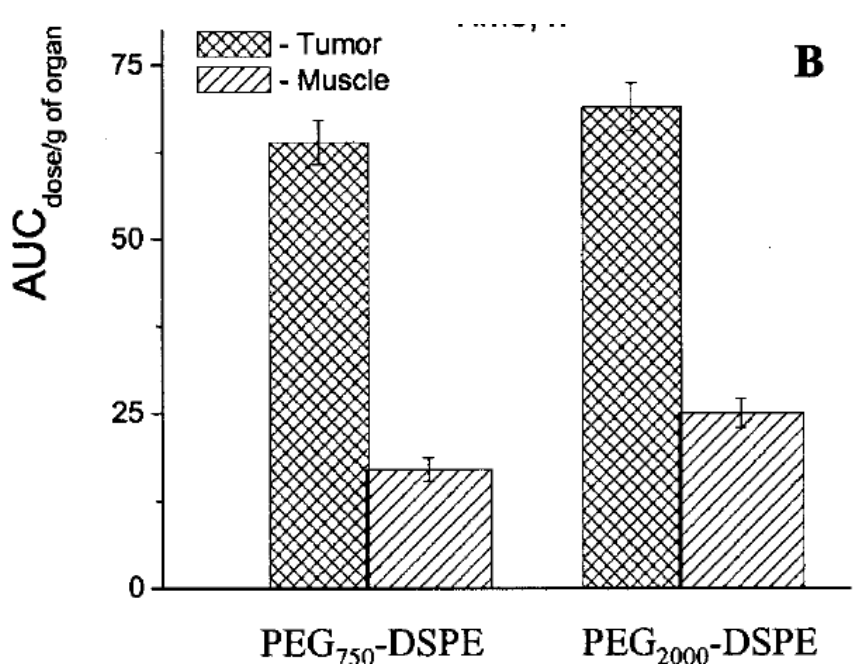
(Gabizon et al. *Clin. Pharmacokin.* 2003)

Enhanced permeation and retention effect in tumors

PEG-diacyllipid*	CMC	Particle size
PEG_{750}-DSPE	1.0×10^{-5} M	7–15 nm
PEG_{2000}-DSPE	1.1×10^{-5} M	7–20 nm

Polyethylene Glycol-Diacyllipid Micelles Demonstrate Increased Acculumation in Subcutaneous Tumors in Mice

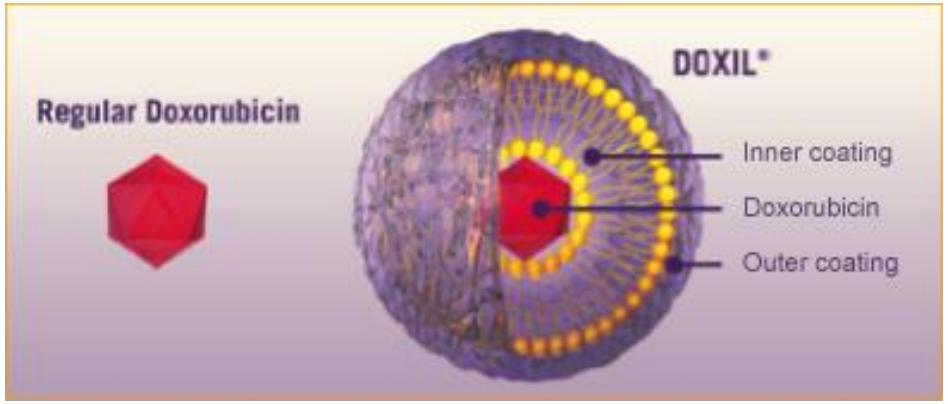




Pharm. Res. **2002**, *19*, 1424-1429.

EPR effect also exist in human patients

Free DOX DOXIL



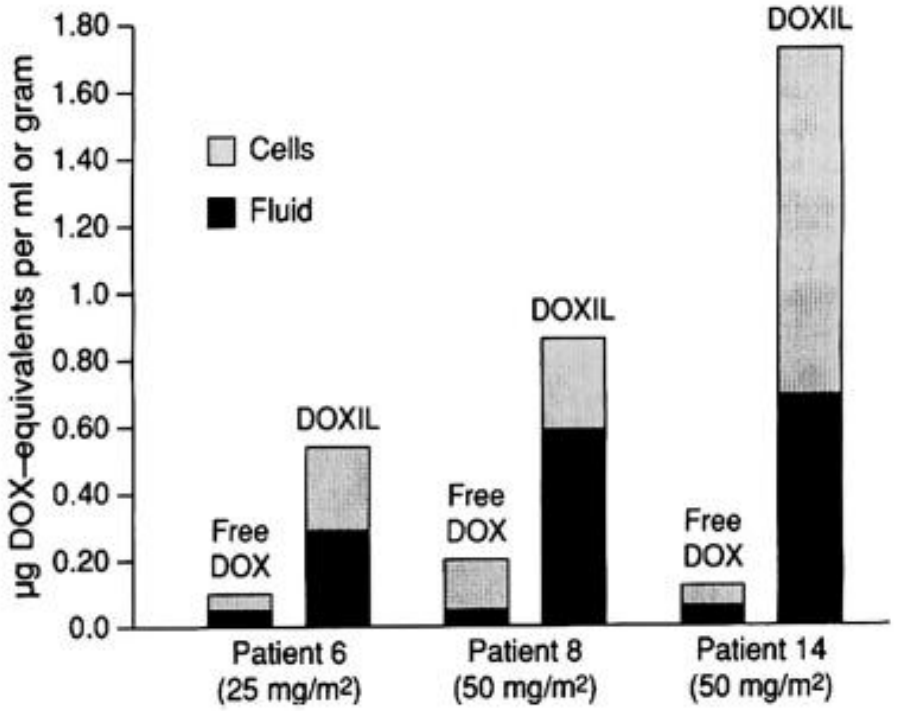
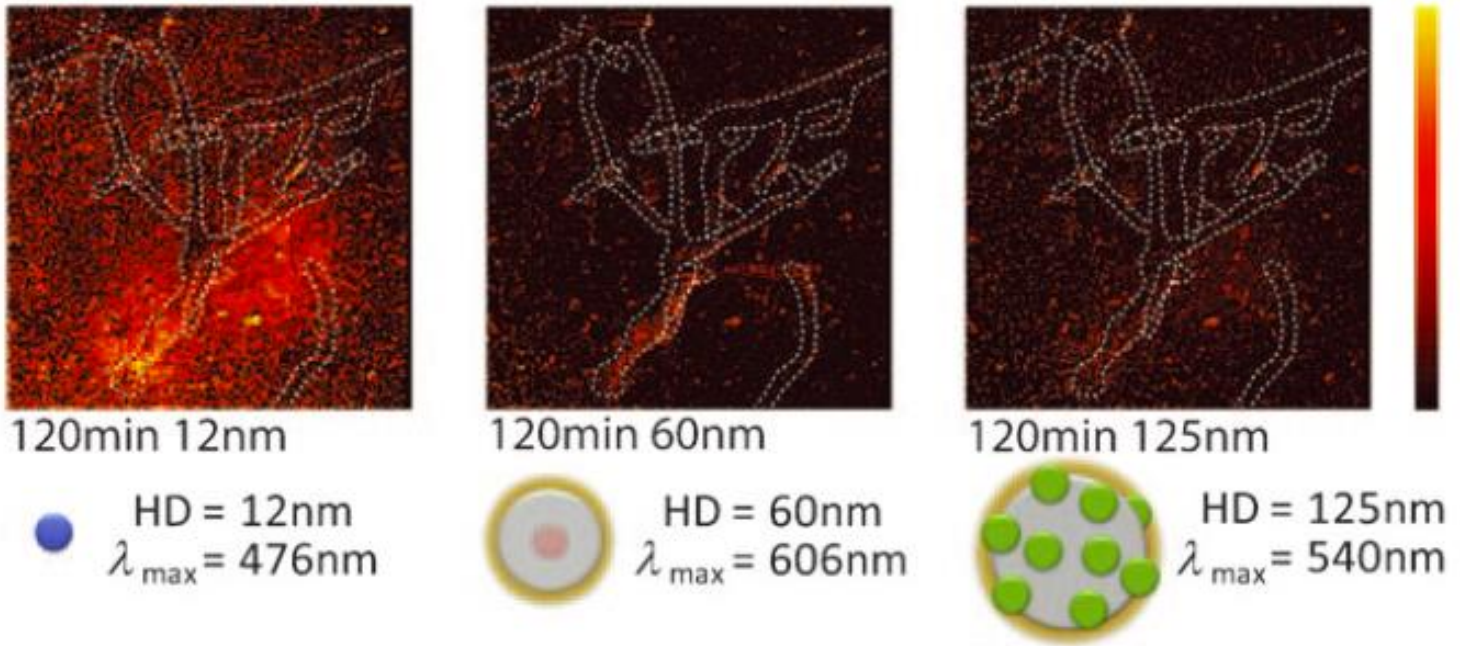
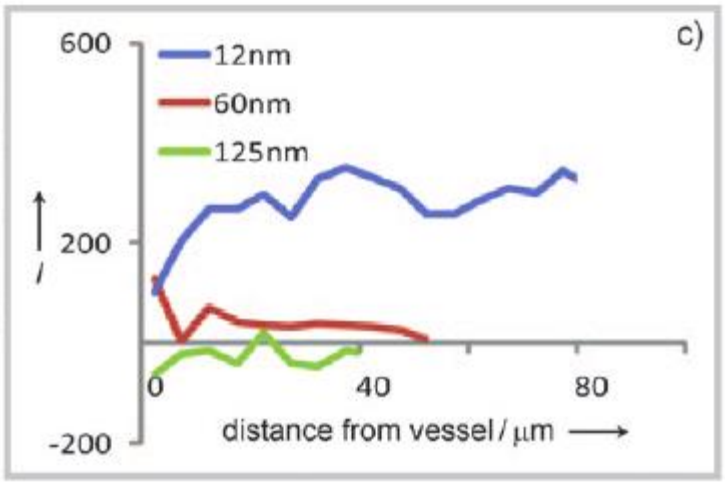


Figure 1. Doxorubicin levels in patients' tumor biopsies, comparing free DOX and DOXIL. Reprinted with permission from ref 26. Copyright 2012 Elsevier Ltd.

Chem. Rev., **2015**, *115* (19), pp 11147–11190

Penetration in tumors: impact of nanoparticle size

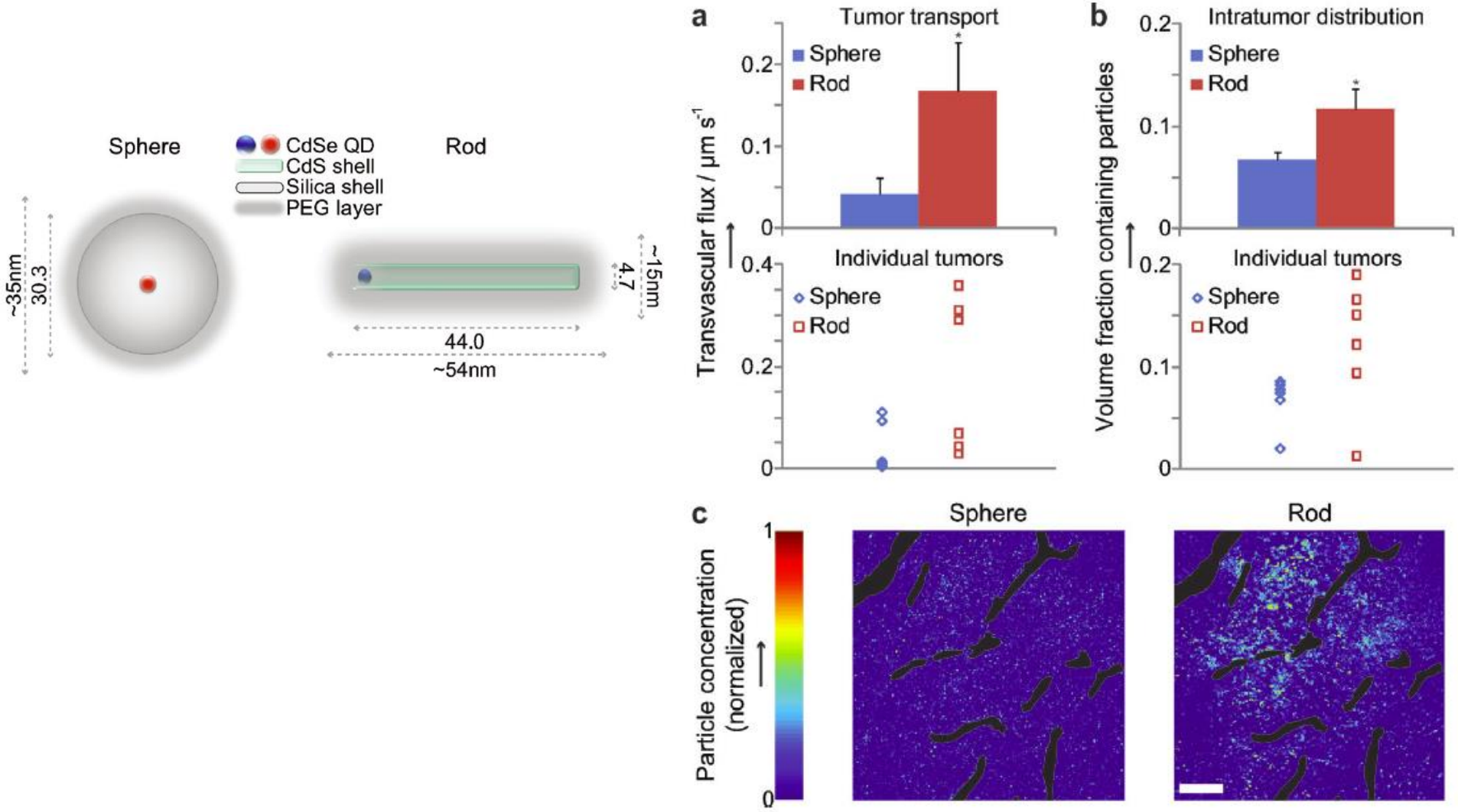




Angew. Chem., Int. Ed. **2010**, *49*, 8649;

This slide is not required.

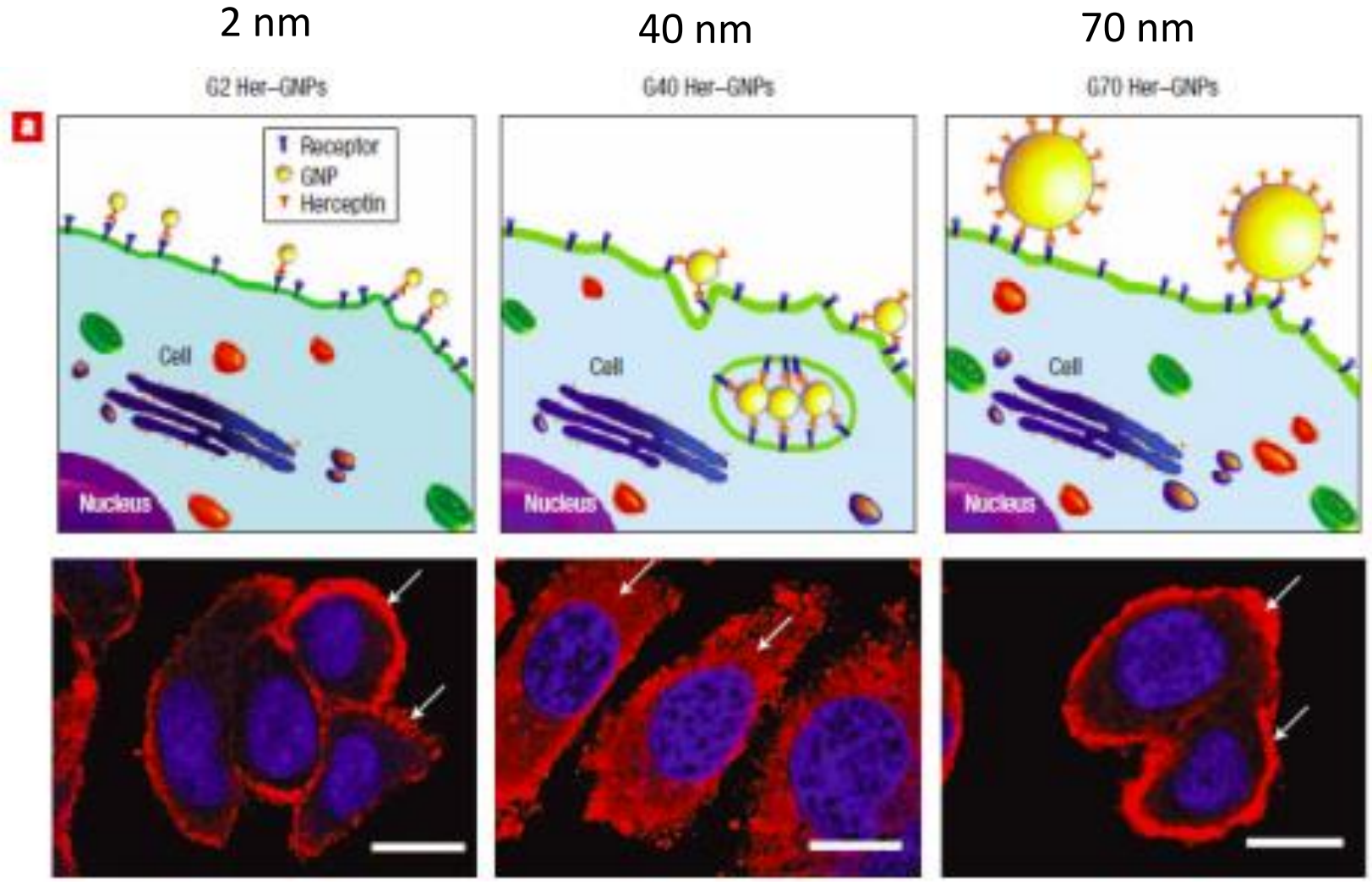
Penetration in tumors: impact of nanoparticle shape



Angew. Chem., Int. Ed. **2011**, *50*, 11417

This slide is not required.

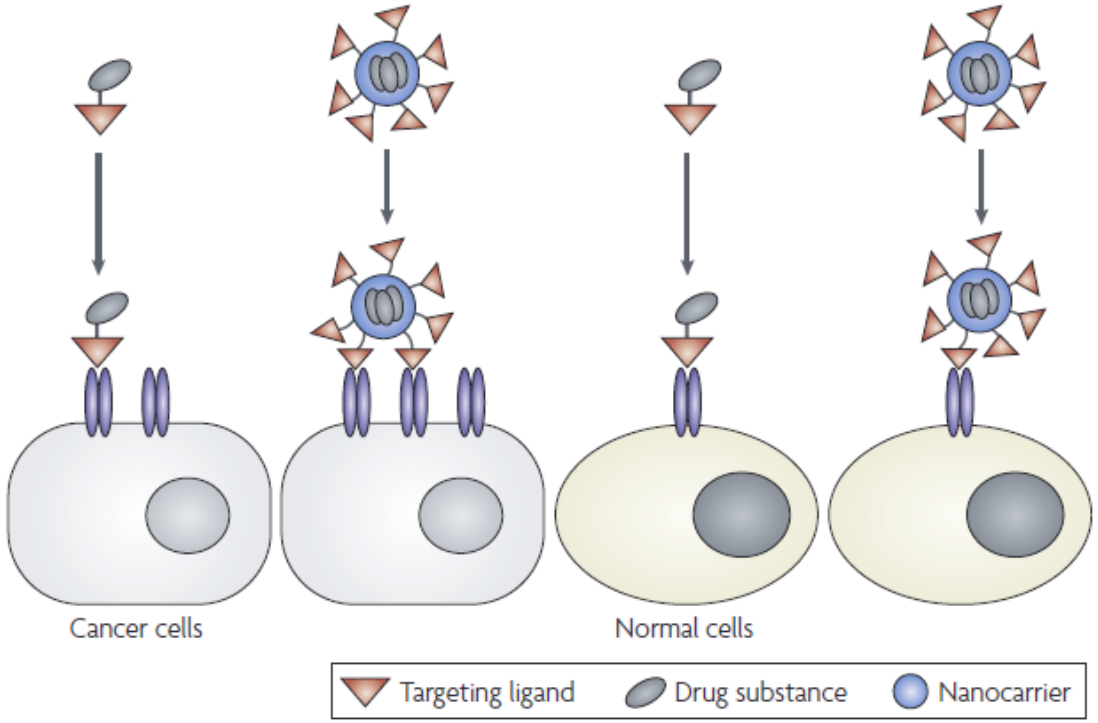
Internalization by tumor cells: impact of nanoparticle size



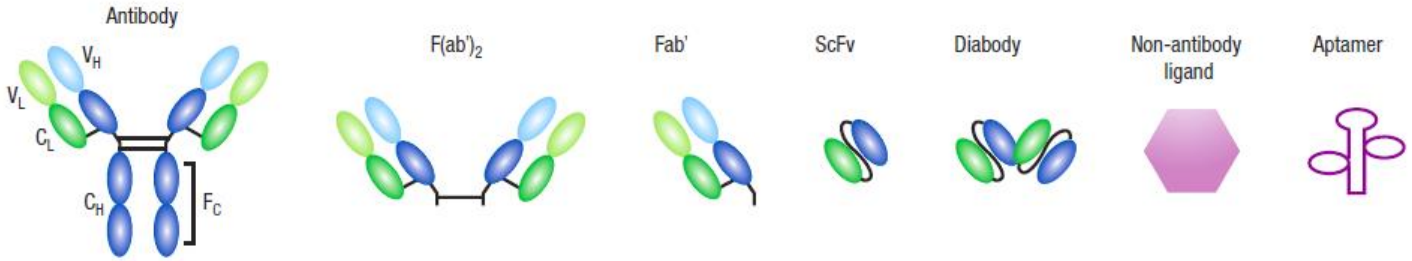
Nat. Nanotechnol. **2008**, *3*, 145; *Proc. Nat. Acad. Sci.* **2008**, *105*, 11613;

This slide is not required.

Active targeting



Nat. Rev. Drug Discov. **7**, 771-782 (2008).



Nat Nanotech, **2007**, 2, 751

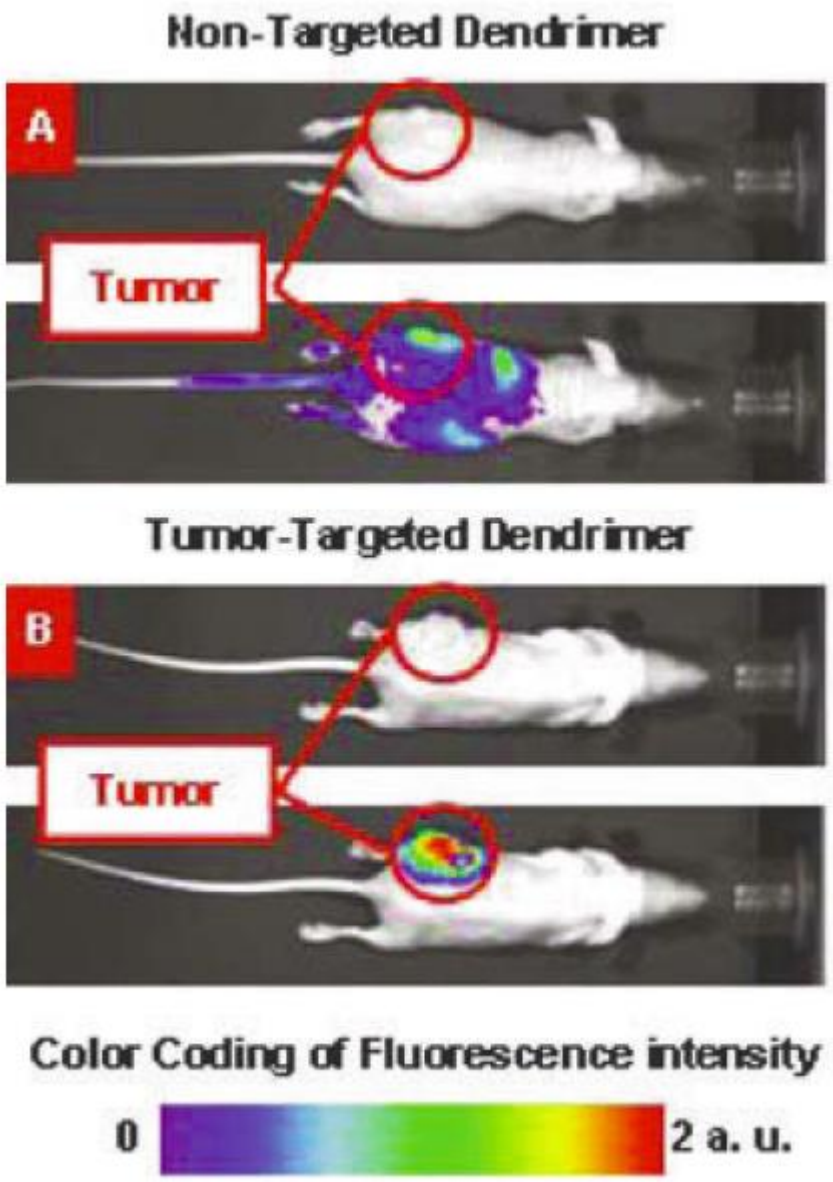
Aptamers (from the Latin aptus – fit, and Greek meros – part) are oligonucleotide or peptide molecules that bind to a specific target molecule.**Aptamers** are usually created by selecting them from a large random sequence pool, but natural **aptamers** also exist in riboswitches.

Table 3 | **Examples of ligand-targeted therapeutic agents**

Name	Targeting agent	Therapeutic agent	Status	Comments	Refs
Gemtuzumab ozogamicin (Mylotarg; UCB/Wyeth)	Humanized anti-CD33 antibody	Calicheamicin	Approved	Antibody–drug conjugate	99
Denileukin diftitox (Ontak; Ligand Pharmaceuticals/Eisai)	Interleukin 2	Diphtheria toxin fragment	Approved	Fusion protein of targeting agent and therapeutic protein	100
Ibritumomab tiuxetan (Zevalin; Cell Therapeutics)	Mouse anti-CD20 antibody	^{90}Yttrium	Approved	Antibody–radioactive element conjugate	101
Tositumomab (Bexxar; GlaxoSmithKline)	Mouse anti-CD20 antibody	^{131}Iodine	Approved	Antibody–radioactive element conjugate	101
FCE28069 (PK2)	Galactose	Doxorubicin	Phase I (stopped)	Small-molecule targeting agent conjugated to polymer nanoparticle	102
MCC-465	F(ab′)$_2$ fragment of human antibody GAH	Doxorubicin	Phase I	Liposome nanoparticle containing antibody fragment targeting agent	103
MBP-426	Transferrin	Oxaliplatin	Phase I	Liposome nanoparticle containing human transferrin protein targeting agent	104,105
SGT-53	Antibody fragment to transferrin receptor	Plasmid DNA with p53 gene	Phase I	Liposome nanoparticle containing antibody fragment targeting agent	106
CALAA-01	Transferrin	Small interfering RNA	Phase I	Polymer-based nanoparticle containing human transferrin protein targeting agent	71,107

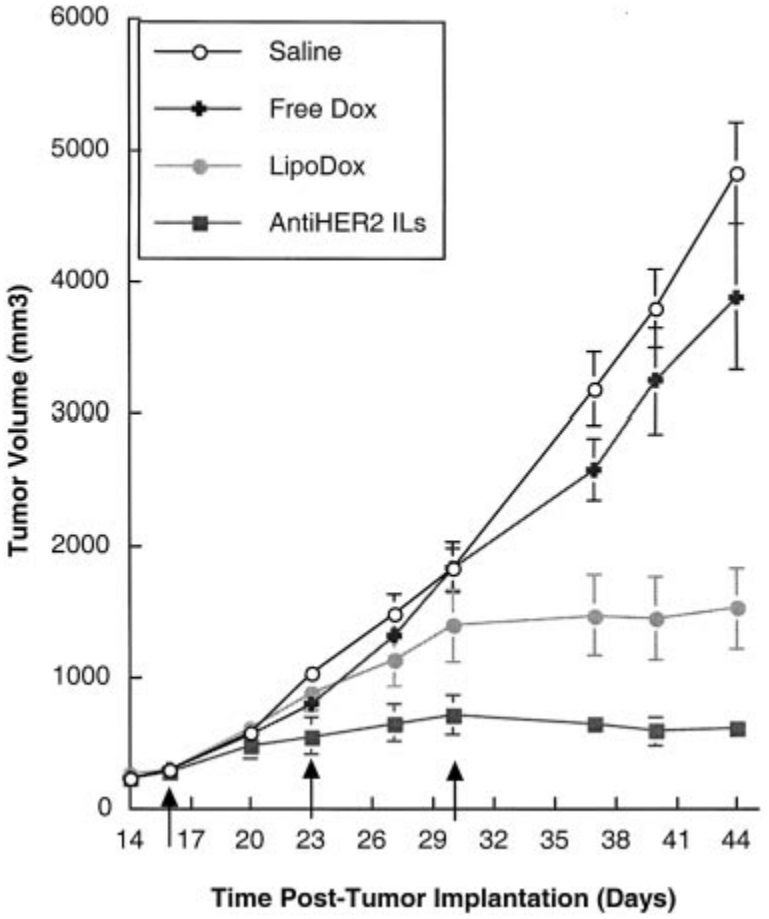
Nat. Rev. Drug Discov. **7**, 771-782 (2008).

Active targeting

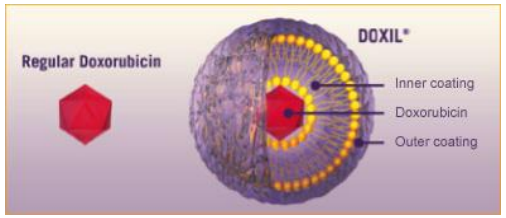


Kularatne, S. A.; Low, P. S. *Methods Mol Biol* 624:249-65 (2010).

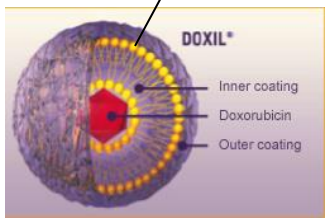
- HER2-positive breast cancer is a breast cancer that tests positive for a protein called **human epidermal growth factor receptor 2 (HER2)**, which promotes the growth of cancer cells.
- HER2 is the target of the monoclonal antibody trastuzumab (marketed as Herceptin).



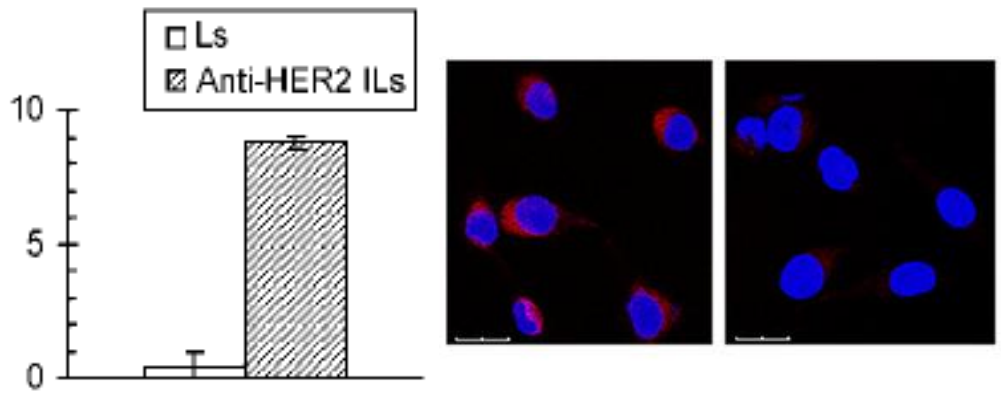
Clin Cancer Res April 1 2002 (8) (4) 1172-1181



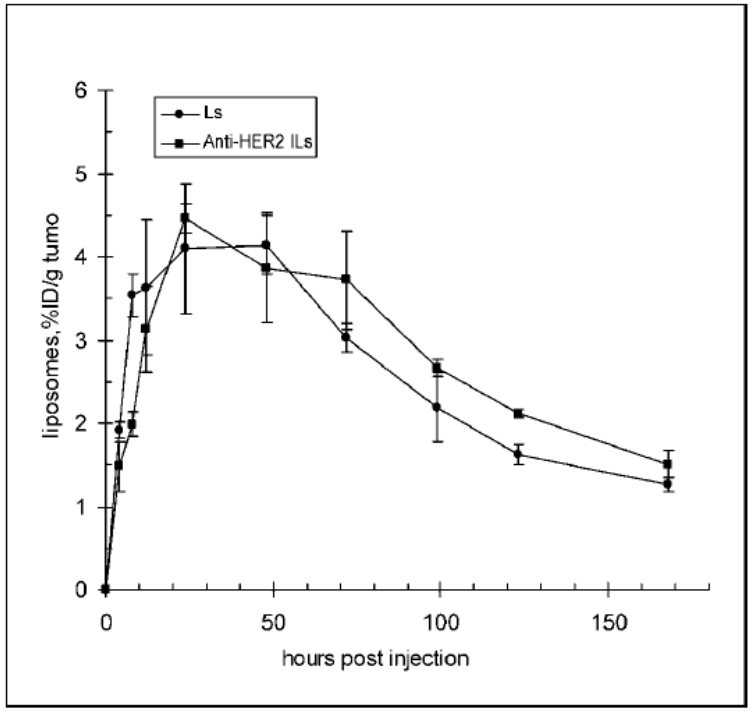
Anti-HER2



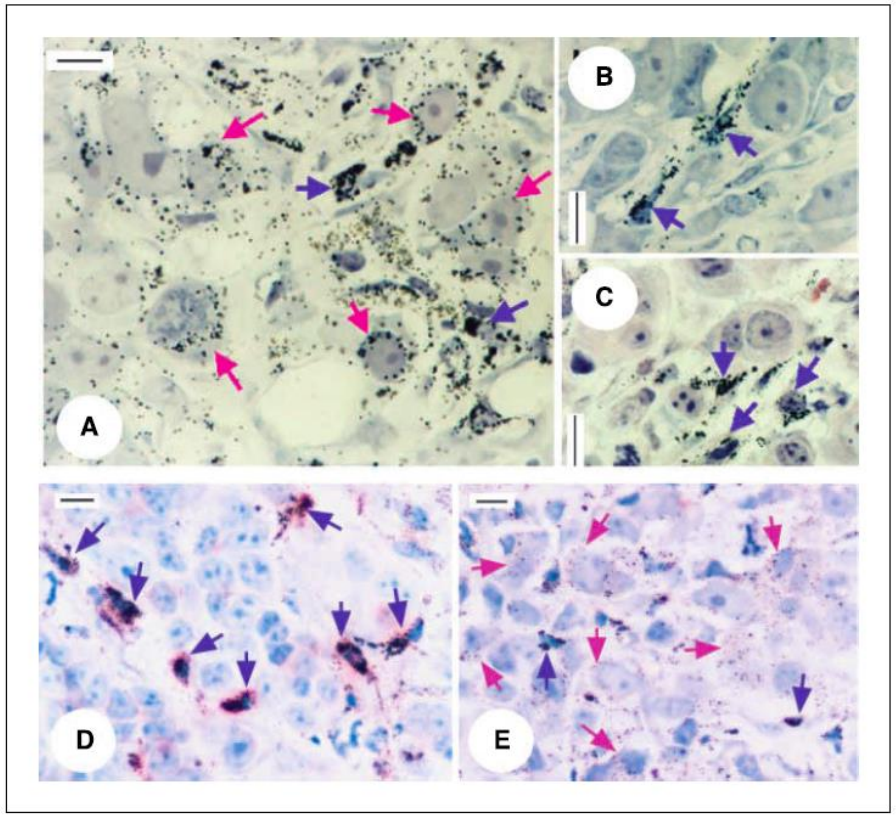
Contradictory results?



Scientific Reports **6**, Article number: 27421 (2016)



Cancer Res. **66**, 6732-6740 (2006).

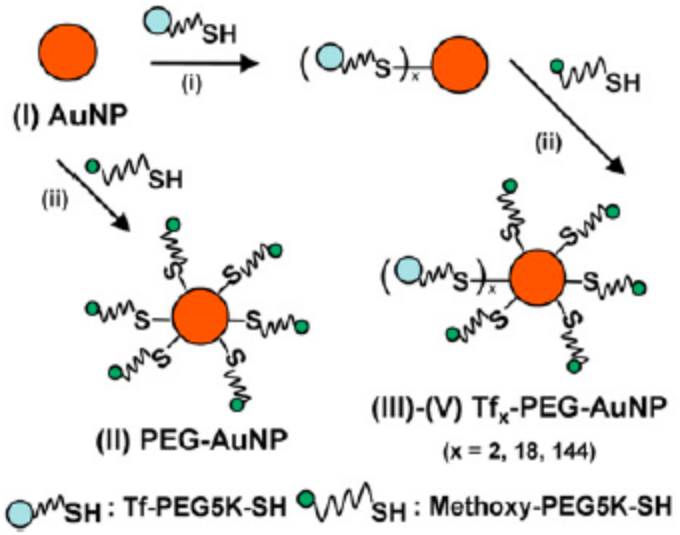


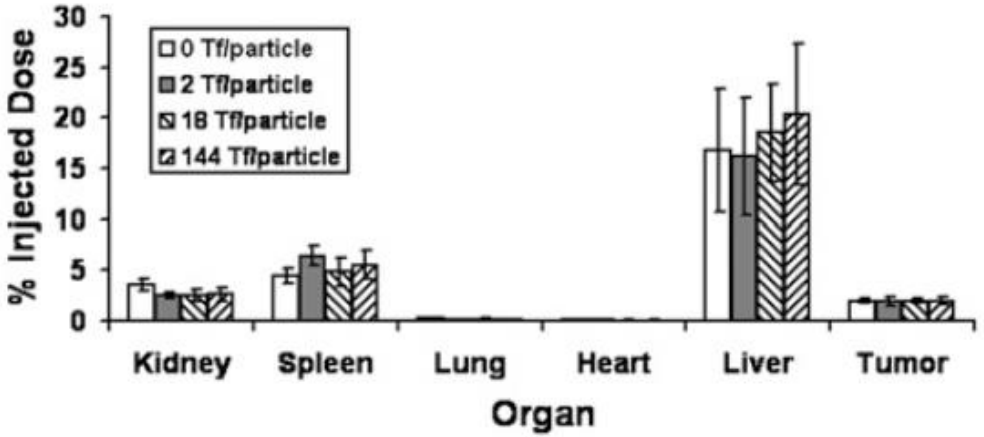
Colloidal gold-encapsulating immunoliposomes/liposomes were administered i.v. in nude mice bearing either HER2-overexpressing BT-474 tumors (A, B, and E)

nonoverexpressing MCF-7 tumors (C and D)

This slide is not required.

Contradictory results?



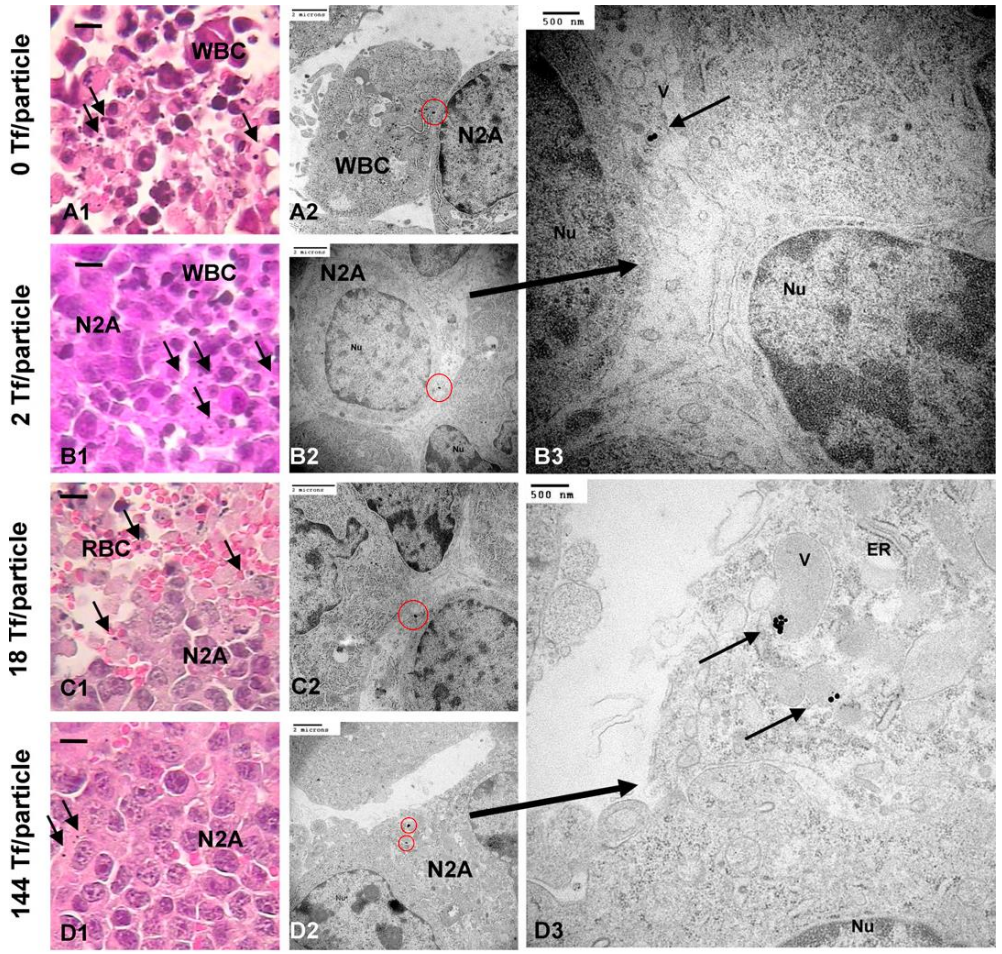


Proc. Natl. Acad. Sci. **107**, 1235-1240 (2010).

This slide is not required.

Contradictory results?

In tumor tissue, the content of targeting ligands significantly influences the number of nanoparticles localized within the cancer cells.



In vivo tumor tissue and intracellular distribution.

(A–D1) Light micrographs of“silver-enhanced” tumor sections.
Arrows indicate “silver enhanced” AuNPs. (Scale bar, 10 µm.) Independent of Tf content, most particles resided near leukocytes. Electron micrographs show particles either engulfed by leukocytes (A2) or tangentially touching Neuro2A cells

(B–C2; enlarged image, B3).(D2 and enlarged image, D3)
Particles with a high Tf content (V: 144 Tf per particle) can enter Neuro2A cells.

ER, endoplasmic reticulum; M, mitochondrion;
N2A, Neuro2A cell; Nu, nucleus;
RBC, red blood cell; V, vesicle;
WBC, leukocyte.

This slide is not required.

Proc. Natl. Acad. Sci. **107**, 1235-1240 (2010).