L11: Ligand-Receptor Engineering & Targeted Delivery

May 10, 2018

1. Physical Forces in Ligand-Receptor Recognition

Non-covalent bonds: On a per-bond basis, non-covalent bonds are 1~3 orders of magnitude weaker than covalent bonds. There are at least five types of non-covalent bonds contribute to ligand-receptor binding:

(1) Electrostatic bond

Charge-charge interaction

$$E_e = \frac{e^2}{4\pi\varepsilon_0} \cdot \frac{Q_1 Q_2}{\kappa_e r} \cdot e^{-K_{dh} r}$$

e: elementary charge, 1.6×10^{-19} coulombs
ε_0: permittivity constant, 8.85×10^{-12} farad/m
Q_1,Q_2: the numbers of attractant charges
r: distance between the charges
k_e: dielectric constant
(k_e=74.3 in water; k_e=40 in hydrophobic condition
the bond is strengthened in hydrophobic condition)
K_{dh}: Debye-Huckel reciprocal length parameter
(K_{dh} =1/1.25 nm at 150 mM NaCl)

e.g., 2 unit charges separated by 0.3 nm

E_e = 19 zJ (hydrophobic environment, zepto: 10^{-21})

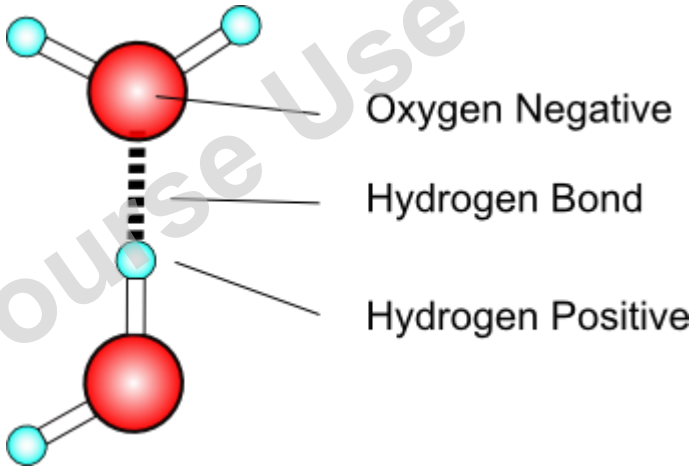
E_e = 10 zJ (pure water)

E_e = 6.3 zJ (1% salt water, 150 mM NaCl)

1. Physical Forces in Ligand-Receptor Recognition

(2) Hydrogen bond

The electrostatic attraction between polar groups that occurs when a hydrogen (H) atom bound to a highly electronegative atom such as nitrogen (N), oxygen (O) or fluorine (F) experiences attraction to some other nearby highly electronegative atom.



2 electronegative atoms share 1 hydrogen atom

Strength per bond: 7~50 zJ

1. Physical Forces in Ligand-Receptor Recognition

(3) Van der Waals interaction

When the electron orbitals of two atoms approach a close distance, there is an attractive component due to the induction of complementary partial charges or dipoles.

$$E_{vdw} = \frac{HA}{12\pi z_{sep}^2}$$

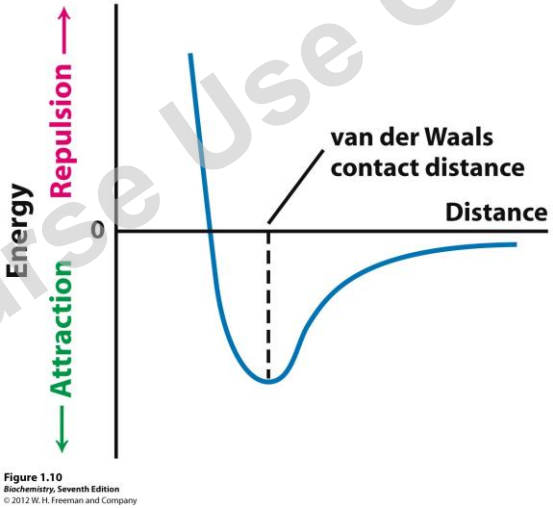


Figure 1.10
Biochemistry, Seventh Edition
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H: Hamaker constant

H = 37 zJ, water
H = 66 zJ, glycerol
H = 340 zJ, diamond

E_{vdw} = 4 zJ, water
E_{vdw} = 8 zJ, small organic molecule

Van der waals bonds are individually very weak, but involve all pairs of neighboring atoms (numerous)

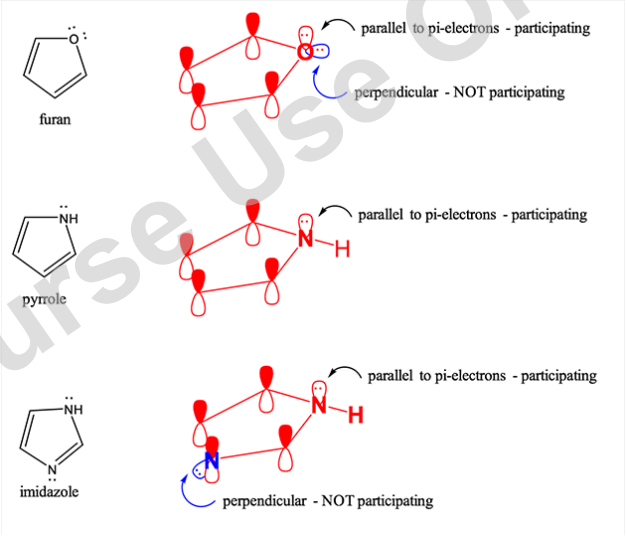
e.g. Fv segment of anti-lysozyme monoclonal antibody has
~ 86 distinct interatomic contact points.

1. Physical Forces in Ligand-Receptor Recognition

(4) Aromatic π bond

Pi electron-to-Pi electron interaction occurs when two aromatic rings approach each other with the plane of their aromatic rings overlapping.

Strength: 40~50 zJ

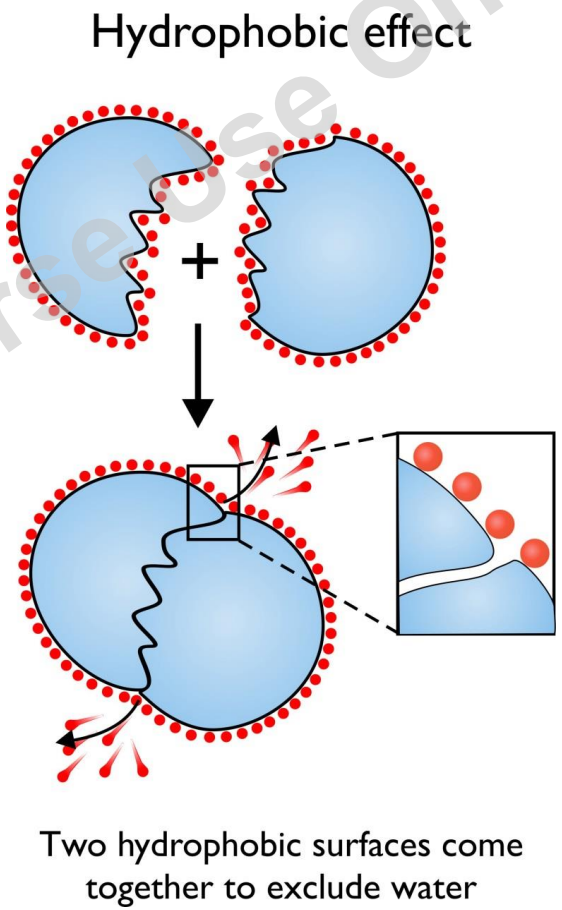


1. Physical Forces in Ligand-Receptor Recognition

(5) Hydrophobic force

When two nonpolar residues approach each other, the surface area exposed to solvent is reduced, increasing the entropy of water molecules while decreasing the entropy of the residues.

Hydrophobic free energy: ~ 17 zJ/nm^2



When designing an artificial binding site, the aforementioned five non-covalent forces may be combined to achieve the desired level of affinity and specificity for a given ligand.

2. Ligand-Receptor Affinity

Affinity: measure the strength of the binding of a ligand to a receptor

$L + R \underset{k_d}{\overset{k_a}{\rightleftharpoons}} L\text{-}R$

k_a: association rate constant (nm^3/molecule·sec)
k_d: dissociation rate constant (sec^{-1})

$K_d = k_d / k_a$ (molecule/nm^3, M)

Half-life of the ligand-receptor complex: $t_{1/2} = \frac{\ln 2}{k_d}$ (sec)

e.g., avidin-biotin binding: $k_d \sim 10^{-7}$ sec^{-1}, $t_{1/2}$ > a few months
enzyme catalase: $k_d \sim 10^{7}$ sec^{-1}, $t_{1/2}$ < 0.1 μs

k_d ⇩ or K_d ⇩ ⟹ $t_{1/2}$ ⇧ Greater affinity
More firmly the receptor grasps the ligand

2. Ligand-Receptor Affinity

The probability $P_{occupied}$ that a receptor will be occupied is given by:

$$P_{occupied} = \left(\frac{C_{ligand}}{K_d}\right) P_{unoccupied}$$

$$P_{unoccupied} = 1 - P_{occupied}$$

To ensure $P_{occupied}$ = 99%, receptor occupancy,

$C_{ligand} \approx 100\ K_d$

e.g. Female serum testosterone, $K_d = 10^{-13}$ molecules/nm^3

$C_{ligand} \approx 10^{-11}$ molecules/nm^3

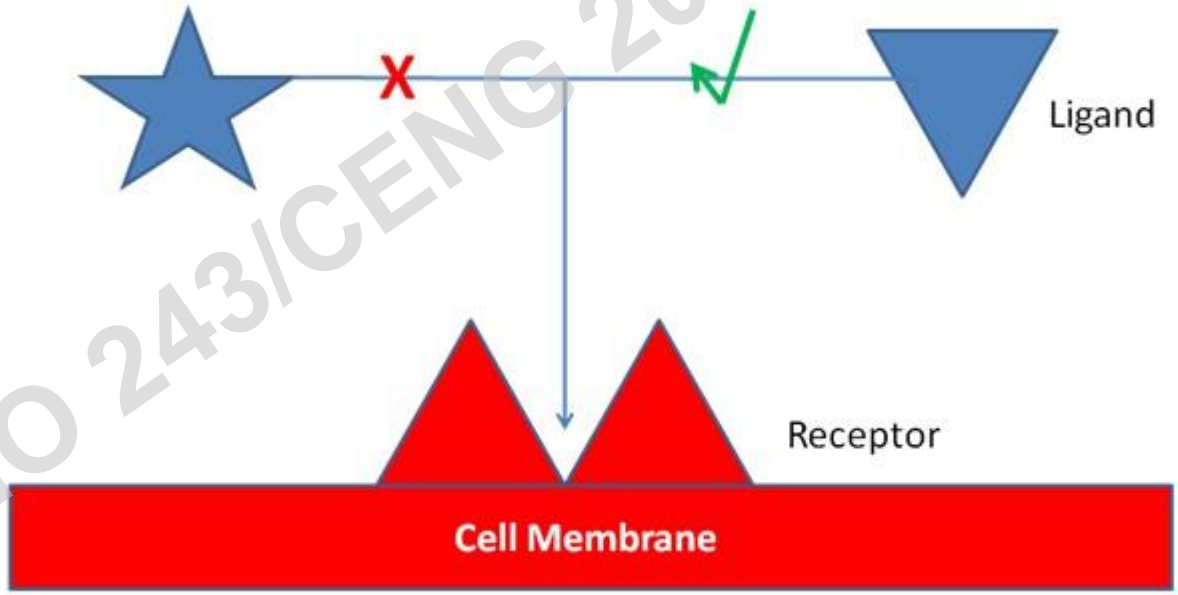
3. Ligand-Receptor Specificity

Specificity: define the degree to which a receptor can distinguish between similar ligands

Affinity of targeted ligand >> Affinity of other ligands

⇧

100~1000 times



4. Typical Ligands and Receptors

Receptors: **the human body contains a minimum of ~ 10^5 distinguishable proteins that can serve as receptors.**

Ligands: **various molecules can serve as ligands**

- Antibodies & Antibody fragments
- Peptides
- Aptamers
- Small molecules (e.g. carbohydrate)

4. Typical Ligands and Receptors

Monoclonal antibodies and antibody fragments

• Methods of preparation: hybridoma technology, antibody phage display, ribosome display and iterative colony filter screening.

• Typical dissociation constants: micromolar to picomolar.

• Remarks: high-affinity monoclonal antibodies can be raised against almost any antigen. Different antibody formats (for example, single-chain variable fragments, Fab fragments, mini-antibodies or immunoglobulin G) show different pharmacokinetics and different tumor-targeting properties.

4. Typical Ligands and Receptors

Peptides

• Methods of preparation: phage display of peptide libraries including in vivo panning, and solid-phase parallel synthesis.

• Typical dissociation constants: micromolar (higher only in exceptional cases, although avidity can be improved by multimerization).

• Remarks: not all antigens lend themselves to the isolation of specific binding peptides of sufficient affinity. The in vivo stability might differ greatly among peptides.

4. Typical Ligands and Receptors

Aptamers

• Methods of preparation: in vitro selection and amplification.

• Typical dissociation constants: micromolar to subnanomolar.

• Remarks: the in vivo stability remains to be properly assessed, even in the case of base analogues. The intriguing possibility exists to generate nuclease-stable ‘Spiegelmers’. The pharmacokinetic properties of the highly charged aptamers (for example, rate of extravasation) remain to be fully characterized.

4. Typical Ligands and Receptors

Small organic molecules

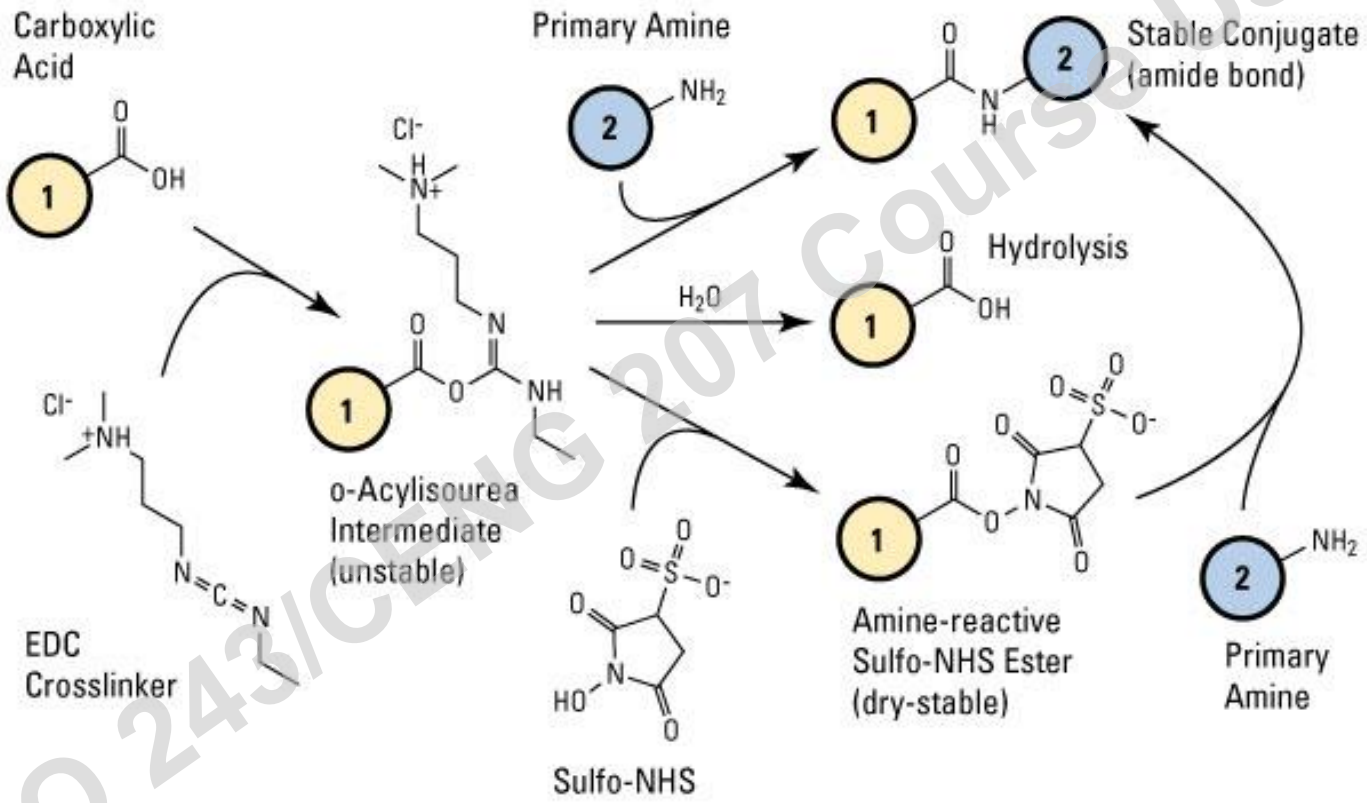
• Methods of preparation: screening of large libraries of compounds, structure–activity relationship by nuclear magnetic resonance, encoded self-assembling chemical libraries, DNA-templated chemistry, dynamic combinatorial chemistry, tethering and speed screen.

• Typical dissociation constants: can be nanomolar for antigens with cavities (for example, enzymes), but isolation of high-affinity small organic binders to flat protein surfaces remains a big chemical challenge.

• Remarks: improvements in the technologies for isolating high-affinity and high-specificity small organic molecules will allow tumor-targeting experiments to be carried out with small molecules with different pharmacokinetic properties. Expected benefits for most small organic binders include easy manufacture, lack of immunogenicity, tissue distribution properties, chemical modification strategies and oral bioavailability.

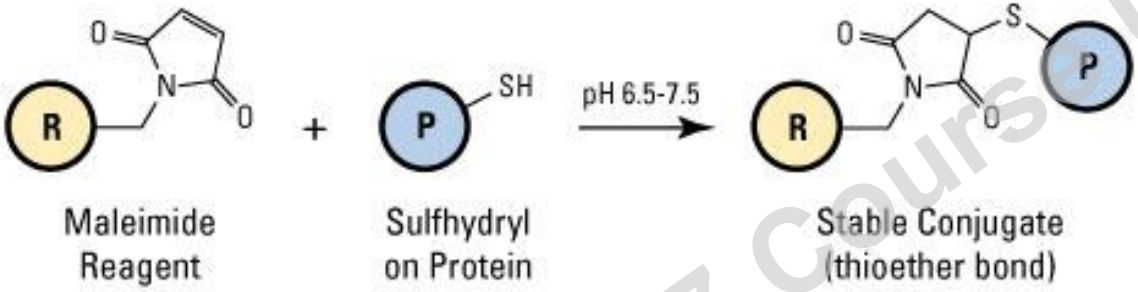
5. Ligand Bioconjugation Chemistry

(1) COOH-NH_2 Reaction

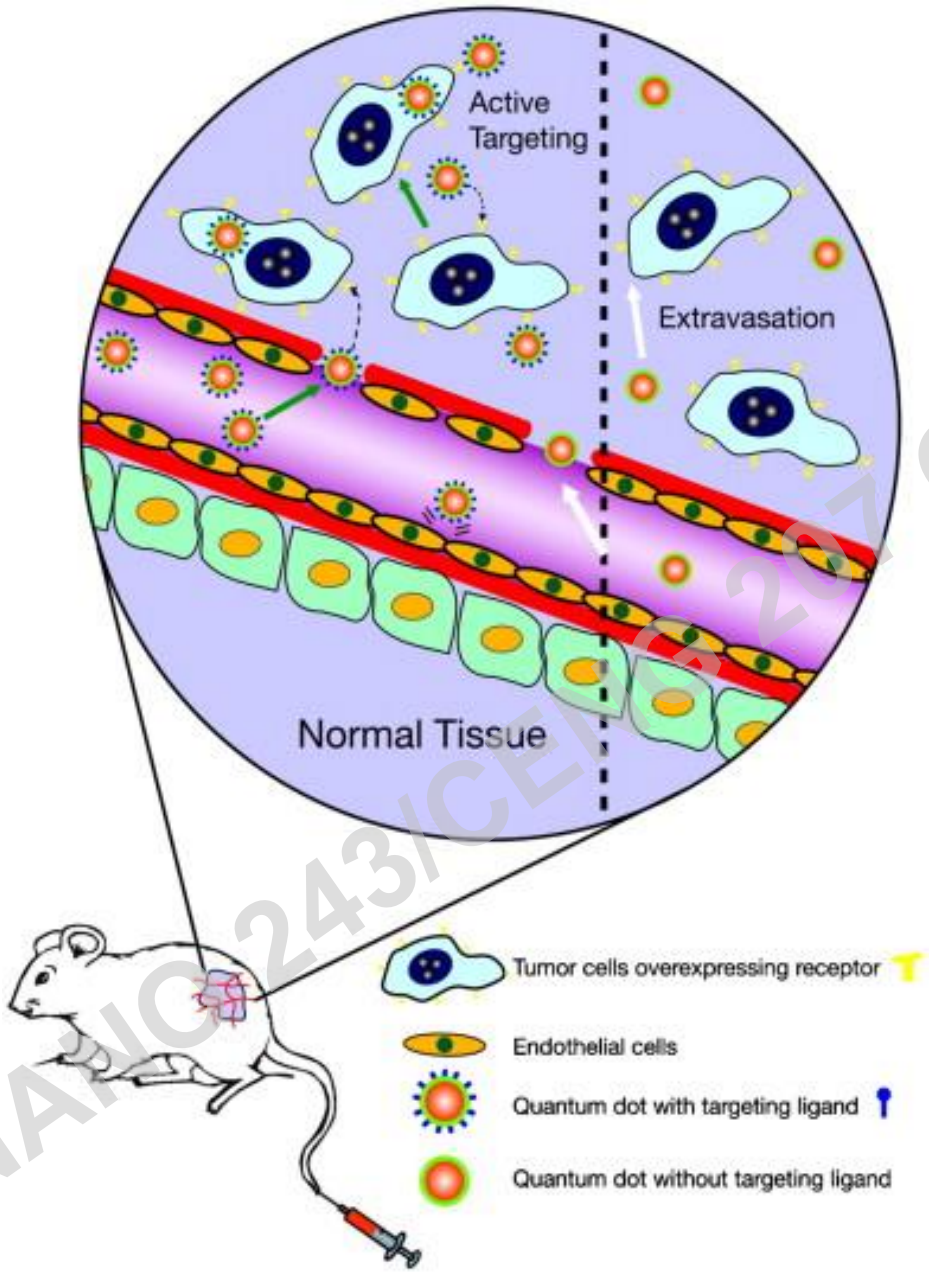


5. Ligand Bioconjugation Chemistry

(2) Maleimide-Thiol Reaction

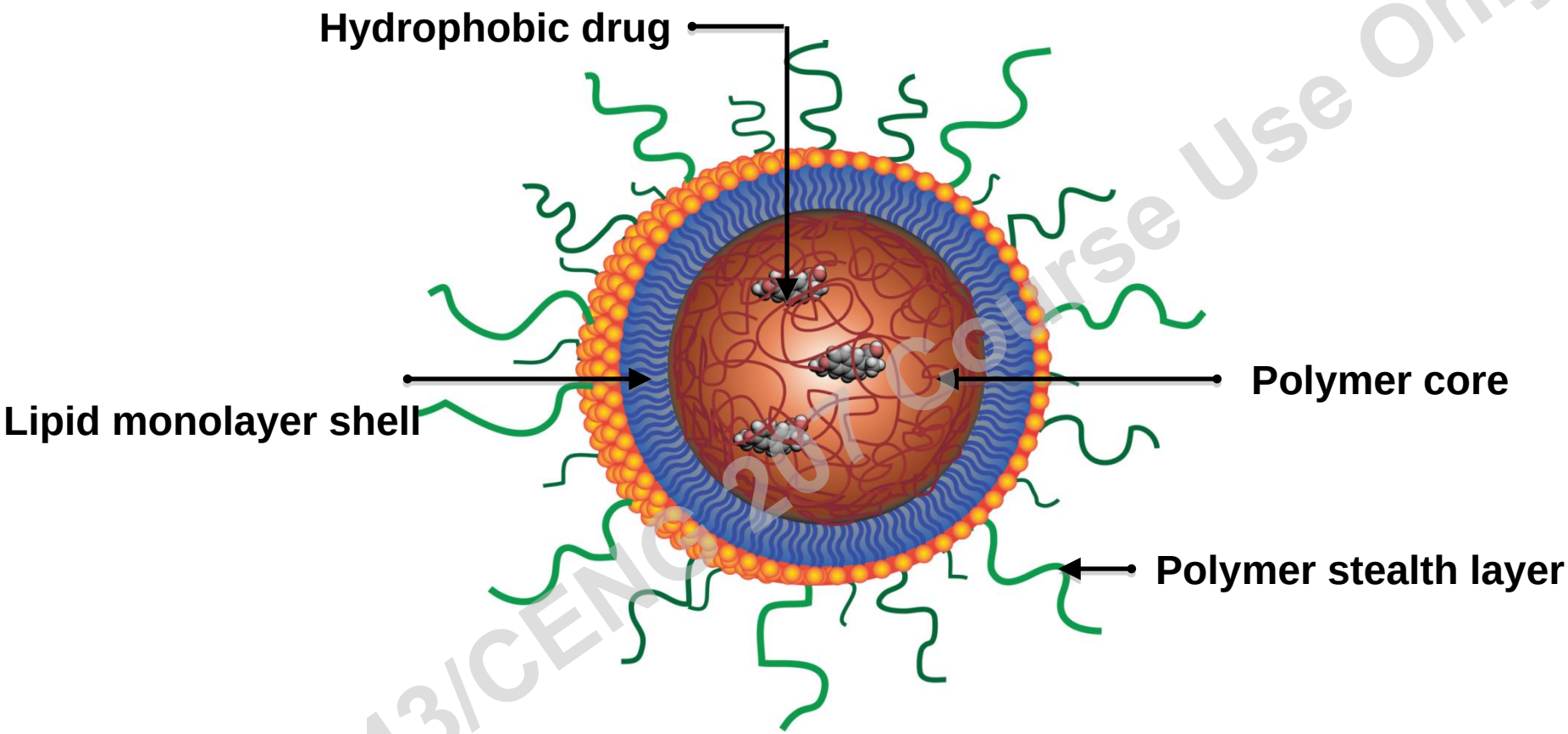


6. Active Tumor Targeting



Smith et al, *Adv. Drug Deliv. Rev.*, 2008

Example 1: Lipid-Polymer Hybrid Nanoparticle



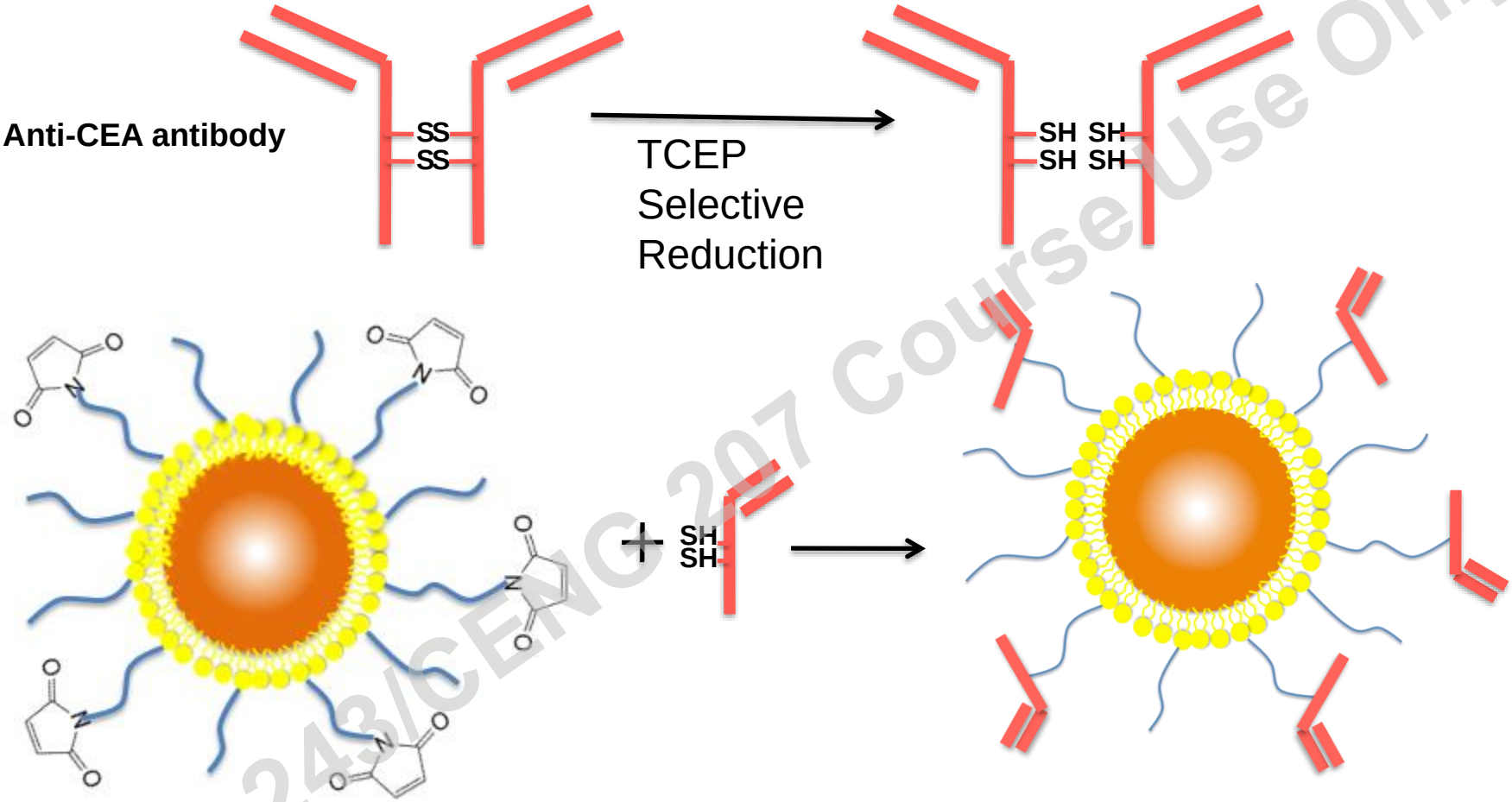
PLGA: Poly lactic-co-glycolic acid

PEG: Polyethylene glycol

Drug: Therapeutic / diagnostic agents

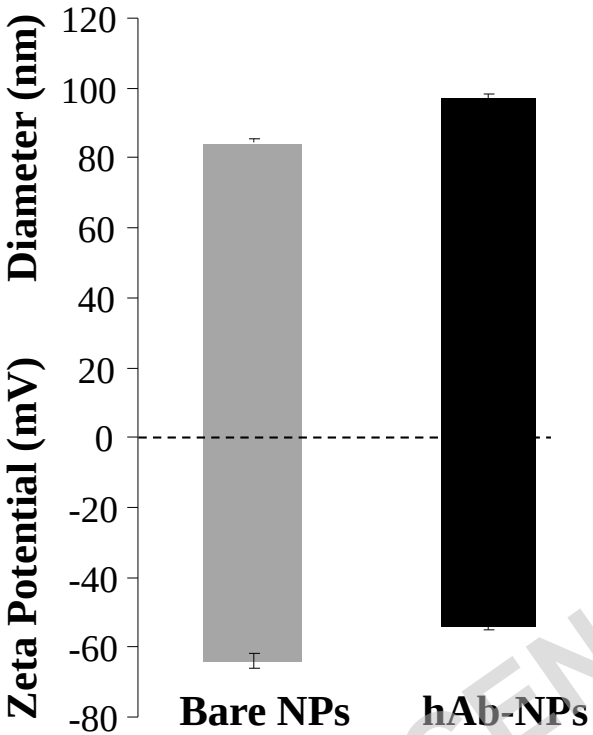
Lipid: Lecithin

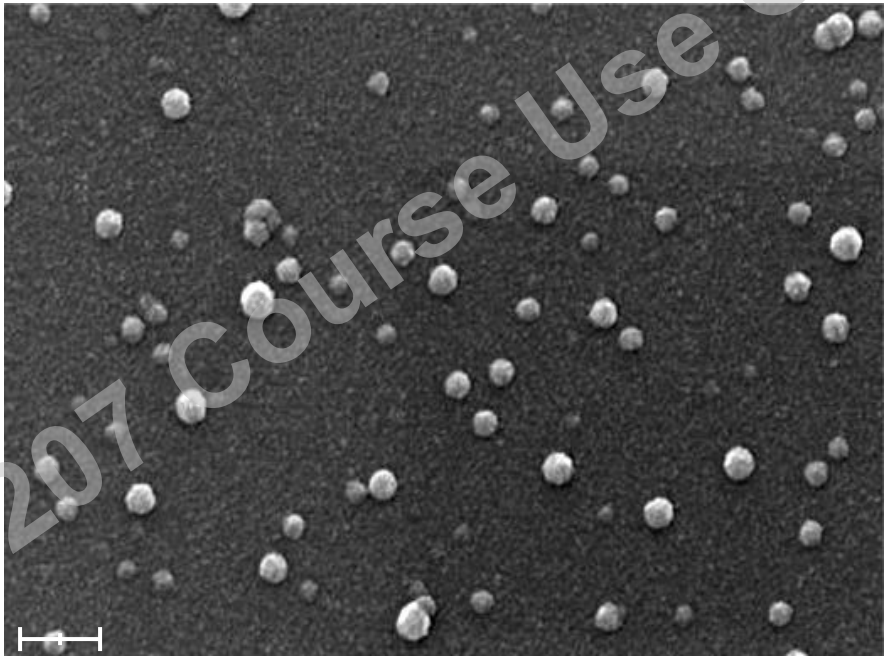
Half-Antibody Targeted Nanoparticles



Advantages: **(i) Site-specific conjugation;**
(ii) Smaller nanoparticle size;
(iii) Simplicity;
(iv) Generic method.

Half-Antibody Targeted Nanoparticles

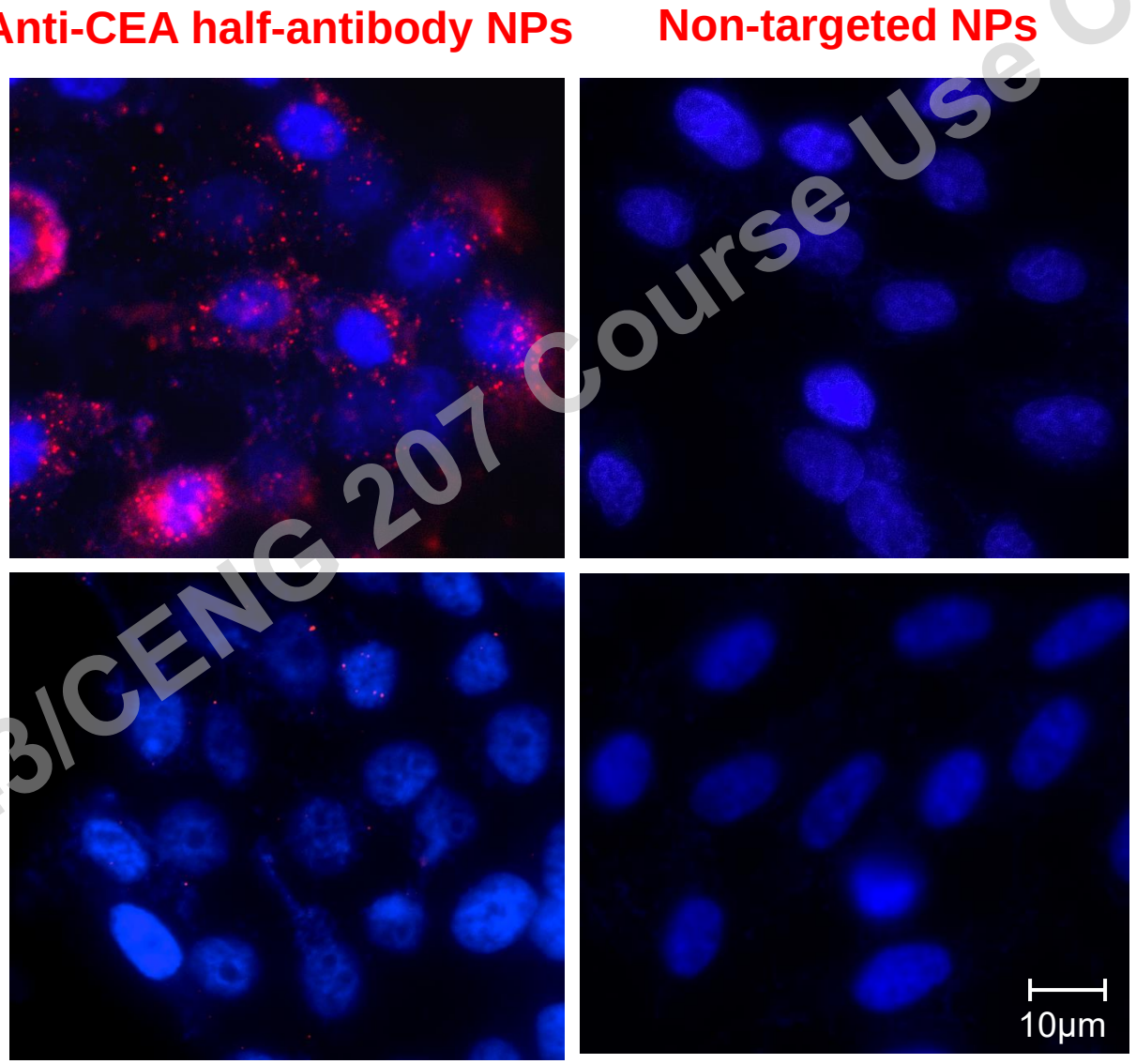




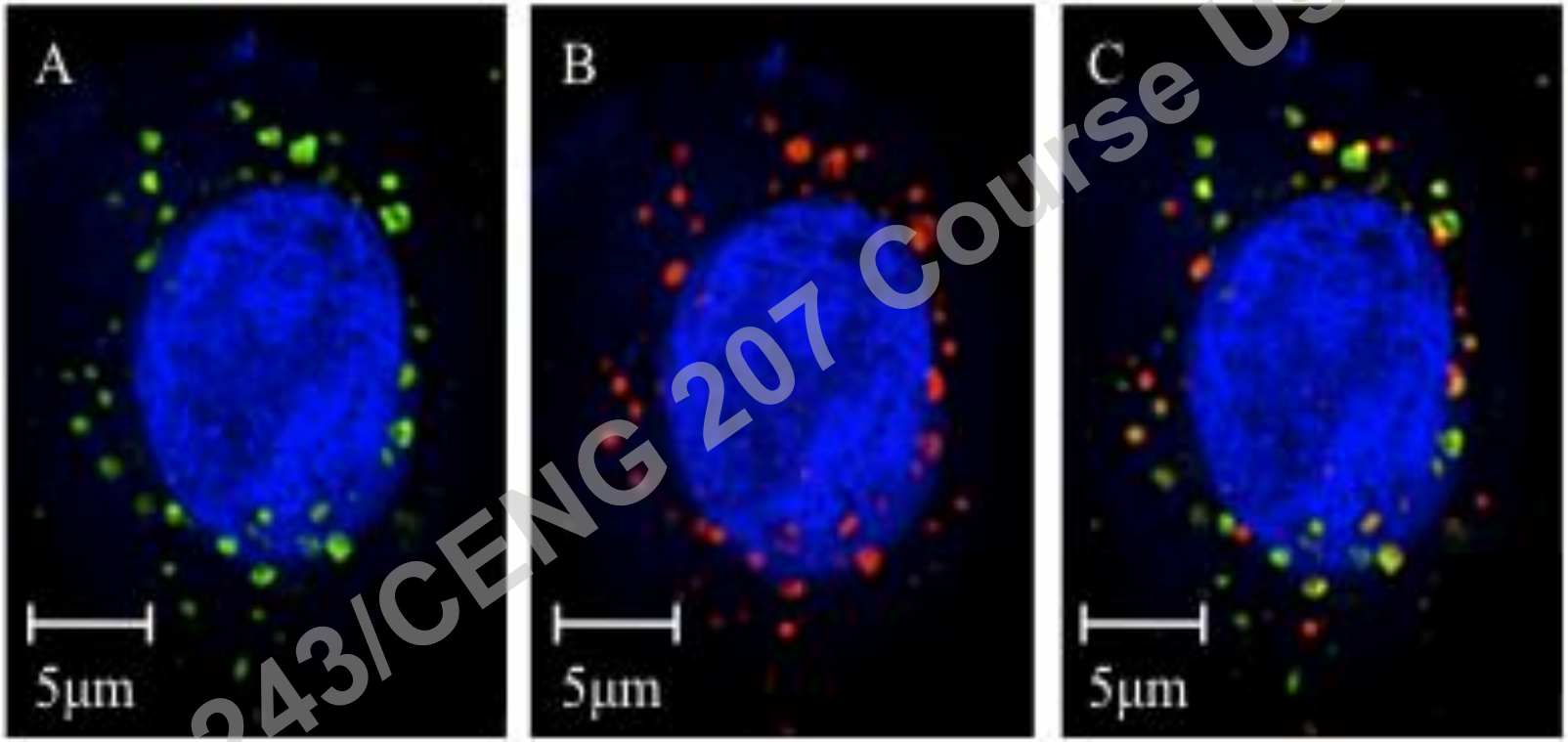
Dynamic light scattering **SEM image**

Size and surface zeta potential of the hybrid NPs before and after hAb conjugation determined by dynamic light scattering. SEM image of hAb targeted lipid-polymer hybrid NPs.

In Vitro Cellular Targeting Ability

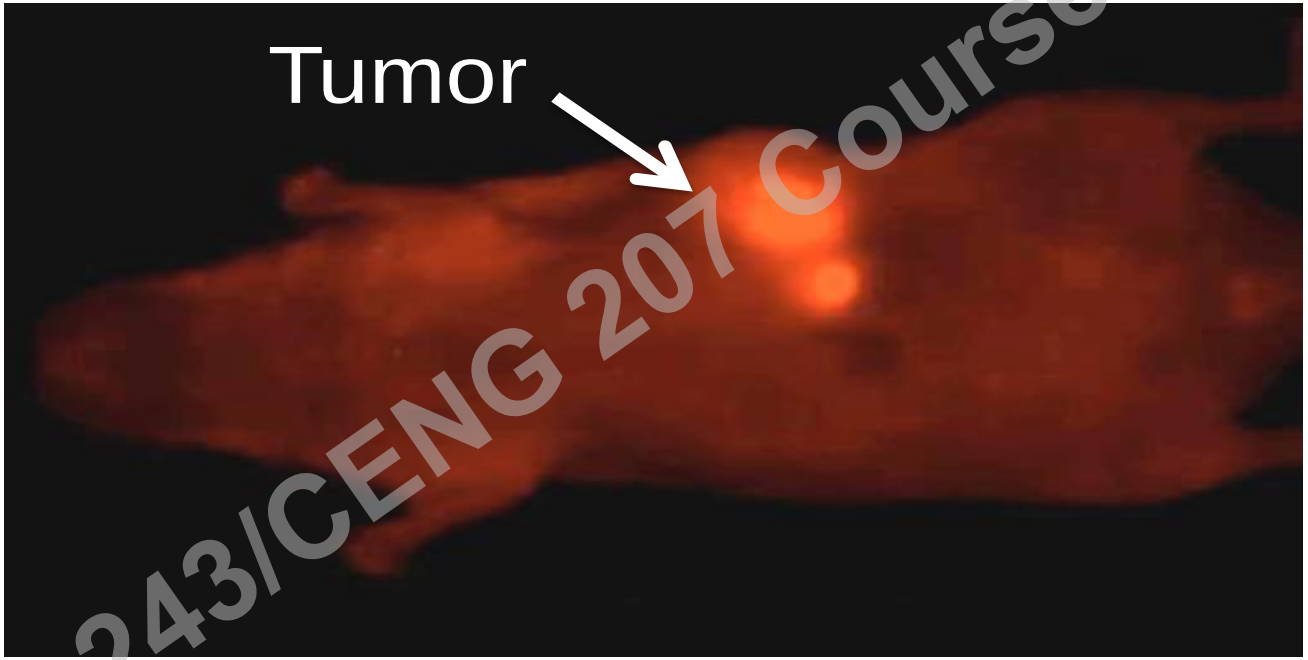


Co-localization Imaging



Hu et al, *Mol. Pharm.*, 2010

In vivo Targeting Ability



7. Active Vascular Targeting

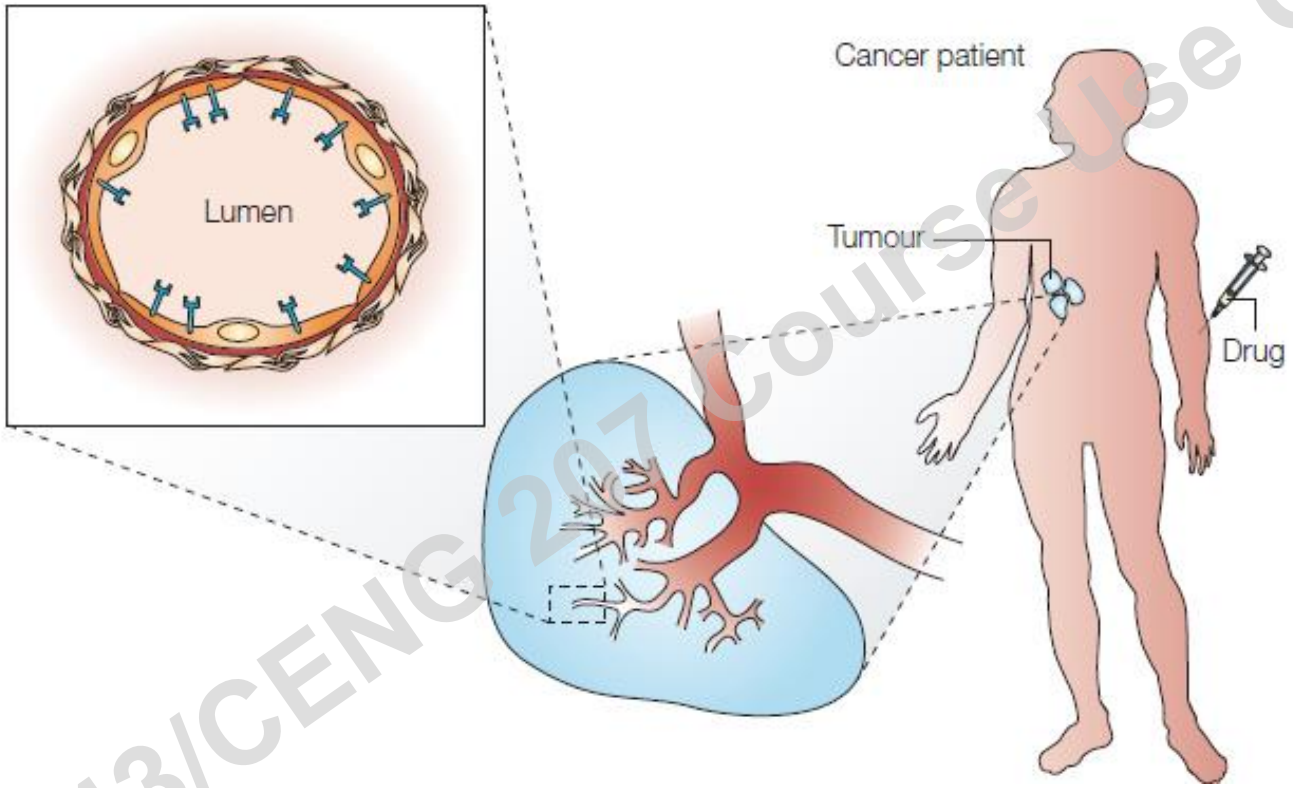
• Proteins that are expressed on the tumor vasculature but not on the vasculature of normal tissues can be used for imaging and targeting purposes.

• Vascular targets include proteins expressed on the endothelial cell, such as DELTA4 or ROBO4, as well as those that are secreted into the stroma around the vessels, such as the differentially spliced isoforms of fibronectin and tenascin.

• Ligands for the vascular targets include antibodies and possibly certain aptamers, such as modified DNA.

• Delivery of a suitable bioactive molecule (toxin, cytokine, etc.) coupled to a targeting ligand can be used to treat solid tumors.

Concept of Tumor Vascular Targeting



The targeted drug is delivered intravenously and homes to the tumour-induced antigen that might be either on the endothelial cell (blue; for example, roundabout-4) or in the perivascular space (red; for example, extra-domain B of fibronectin).

Example 2: NP-mediated tumor vasculature delivery

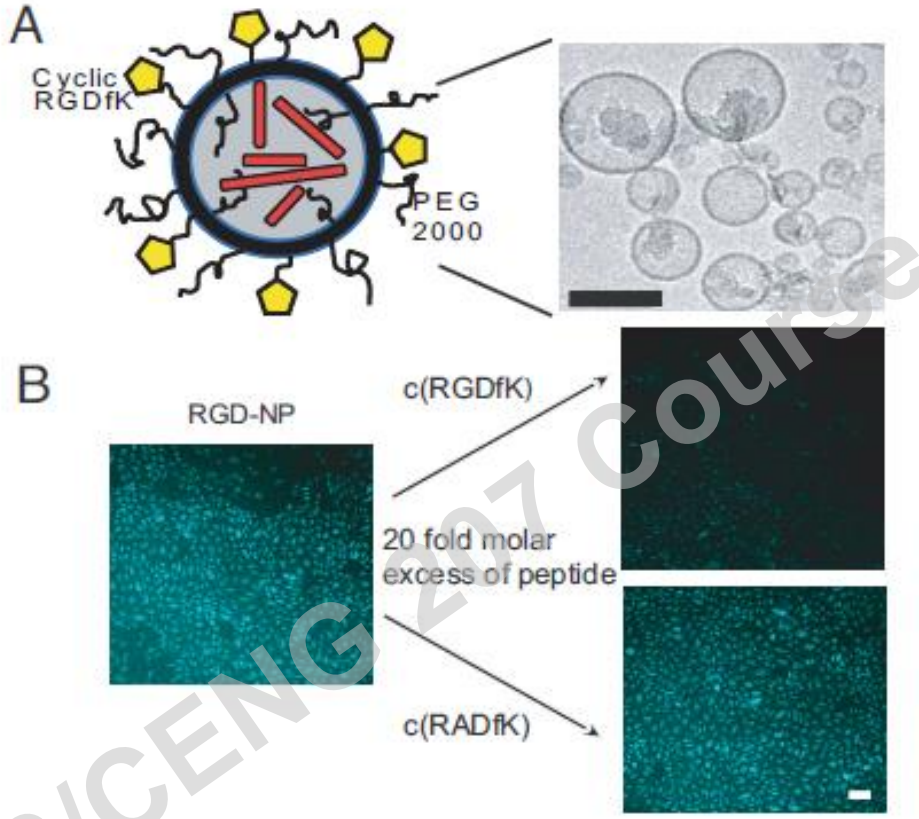
Integrin αvß3 is found on a subset of tumor blood vessels where it is associated with angiogenesis and malignant tumor growth.

We designed an αvß3-targeted nanoparticle (NP) encapsulating the cytotoxic drug doxorubicin (Dox) for targeted drug delivery to the αvß3-expressing tumor vasculature.

We observed real-time targeting of this NP to tumor vessels and noted selective apoptosis in regions of the 3-expressing tumor vasculature.

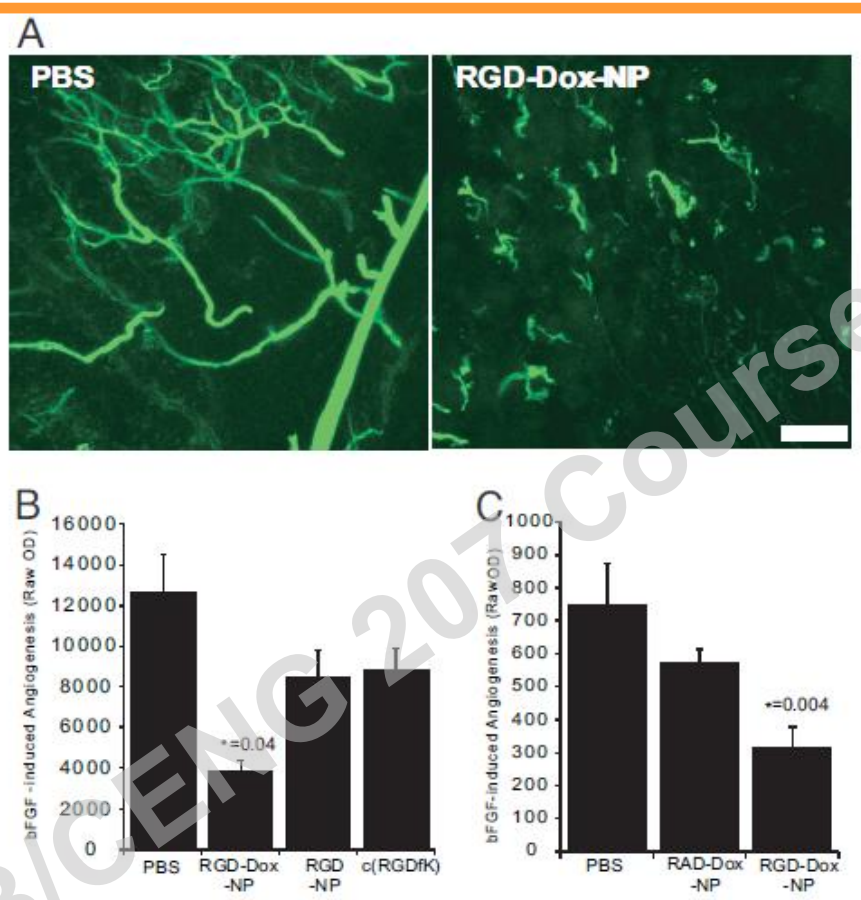
PNAS. 2008, 105, 9343.

NP Design and Characterization



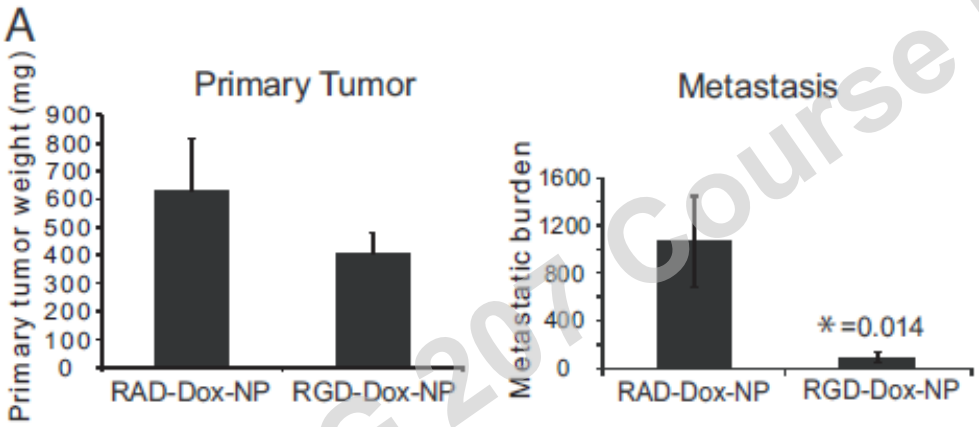
(A) Schematic representation and TEM of Dox-loaded RGD-NP. (B) Competition assay for in vitro $\alpha_v ß_3$ targeting of RGD-NP in endothelial cells. HUVECs were pretreated for 5 min with a 20-fold molar excess of either cRGDfK or cRADfK to test for inhibition of NP binding. Subsequently, the cells were incubated with the RGD-NPs for 20 min, and binding was studied by scanning confocal microscopy for the BODIPY 630/650 dye.

In vivo Disruption of Tumor Vasculature



Vascular disruption in the mouse Matrigel model with αvß3 -targeted RGD-Dox-NP. Mice were injected s.c. on the flank with Matrigel containing 400 ng of human recombinant bFGF. NPs containing 1 mg/kg of Dox were i.v.-injected on days 1, 3, and 5. (A) After the treatment, mice were i.v.-injected with fluorescein-labeled G. simplicifolia lectin and the plugs were removed and imaged by scanning confocal microscopy. (B and C) To quantify angiogenesis, the matrigel plugs were removed and the fluorescent-lectin content was quantified.

In Vivo Efficacy



RGD-Dox-NP reduces overall metastasis in an orthotopic renal cell carcinoma model.

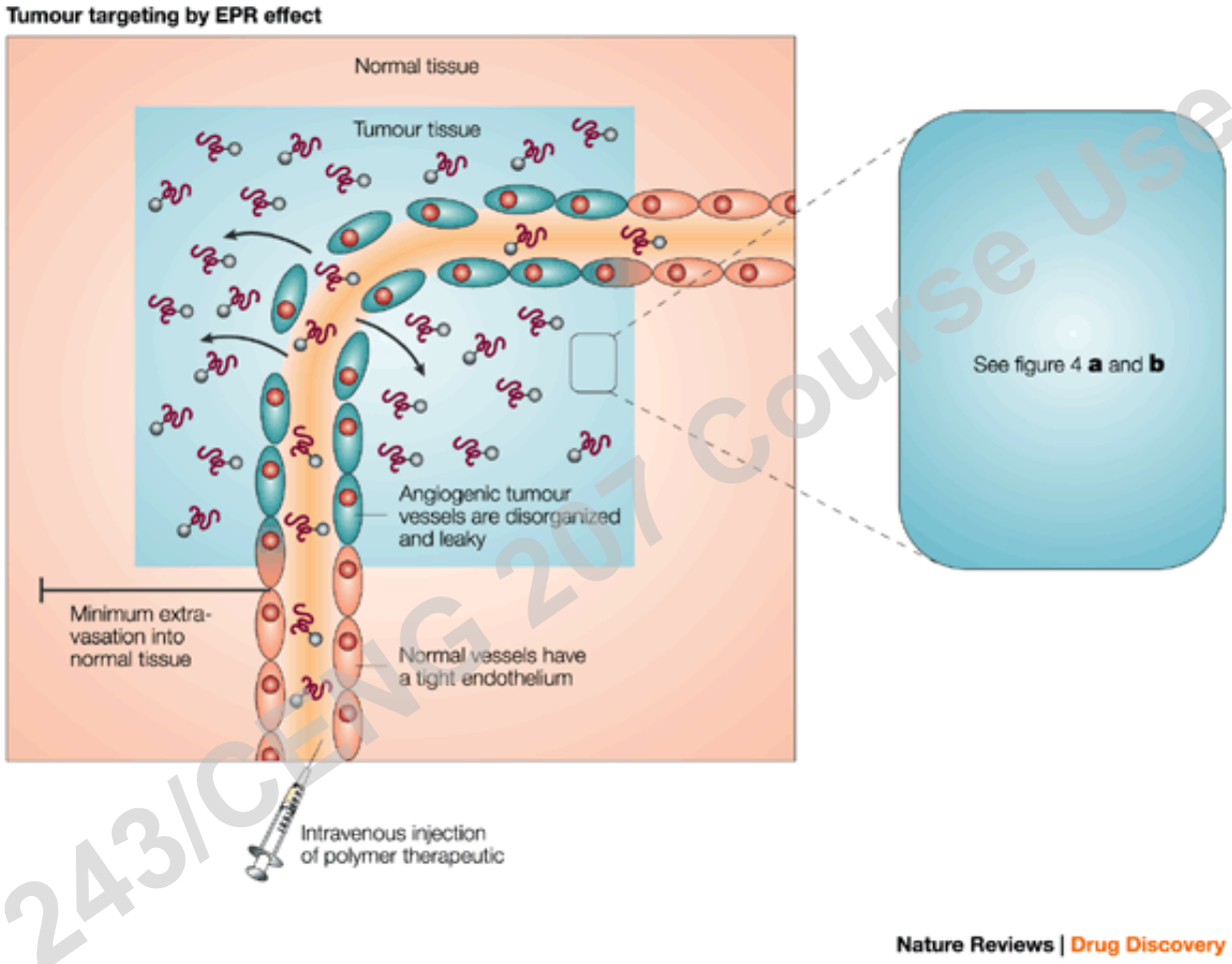
8. Passive Targeting (EPR Effect)

The endothelium (innermost cell layer) of tumor capillaries is less well organized and have numerous and larger pores than normal healthy vessels.

Nanoparticles with a suitable size stay in the blood stream until reaching tumor tissue where they selectively leak into the tumor tissue.

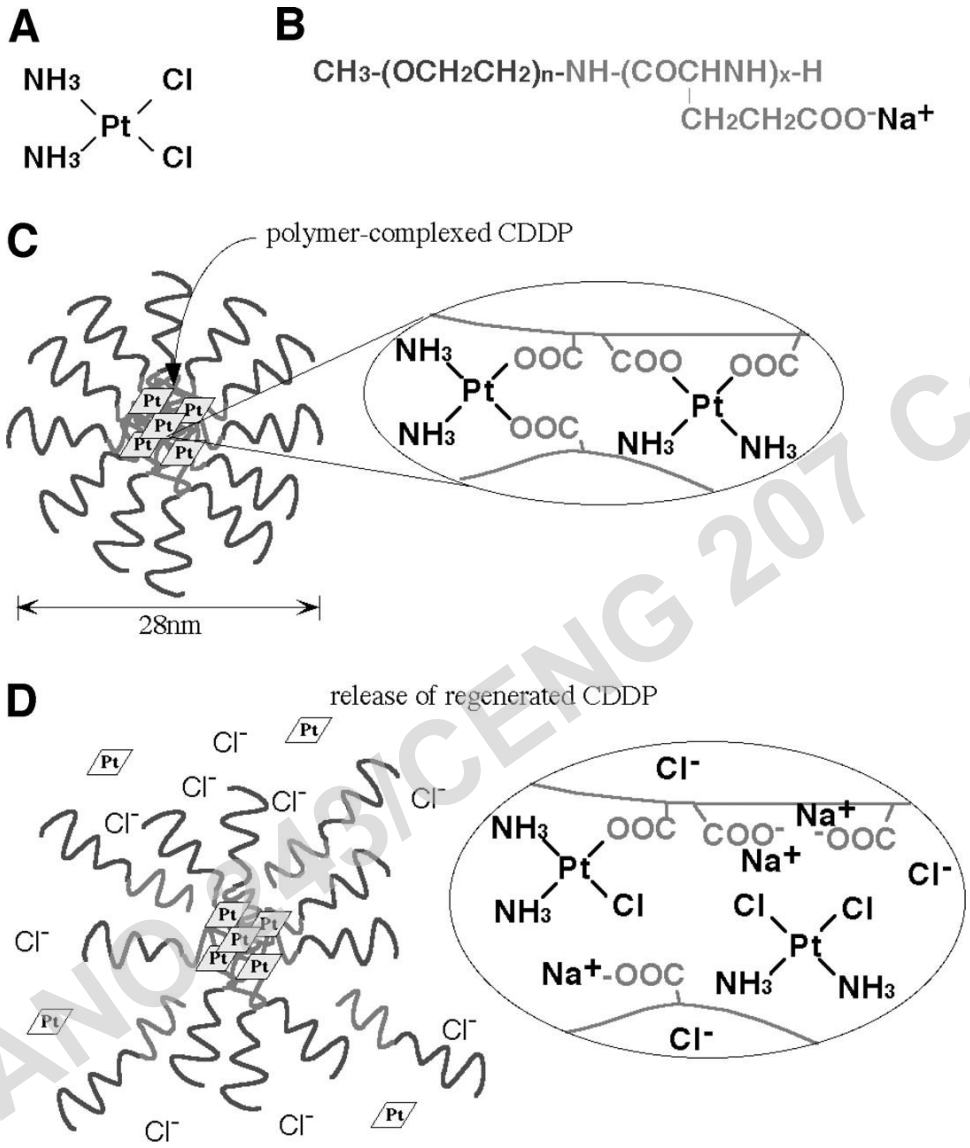
This gives a larger difference in concentration of the nanoparticle-based agents in tumor tissue compared to normal tissue.

Enhanced Permeability and Retention (EPR) Effect



Tumor targeting of long-circulating polymer therapeutics occurs passively by the 'enhanced permeability and retention' (EPR) effect. Hyperpermeable angiogenic tumor vasculature allows preferential extravasation of circulating macromolecules and polymeric micelles. Once present in the tumor interstitium, polymer therapeutics act either after endocytic internalization or extracellularly.

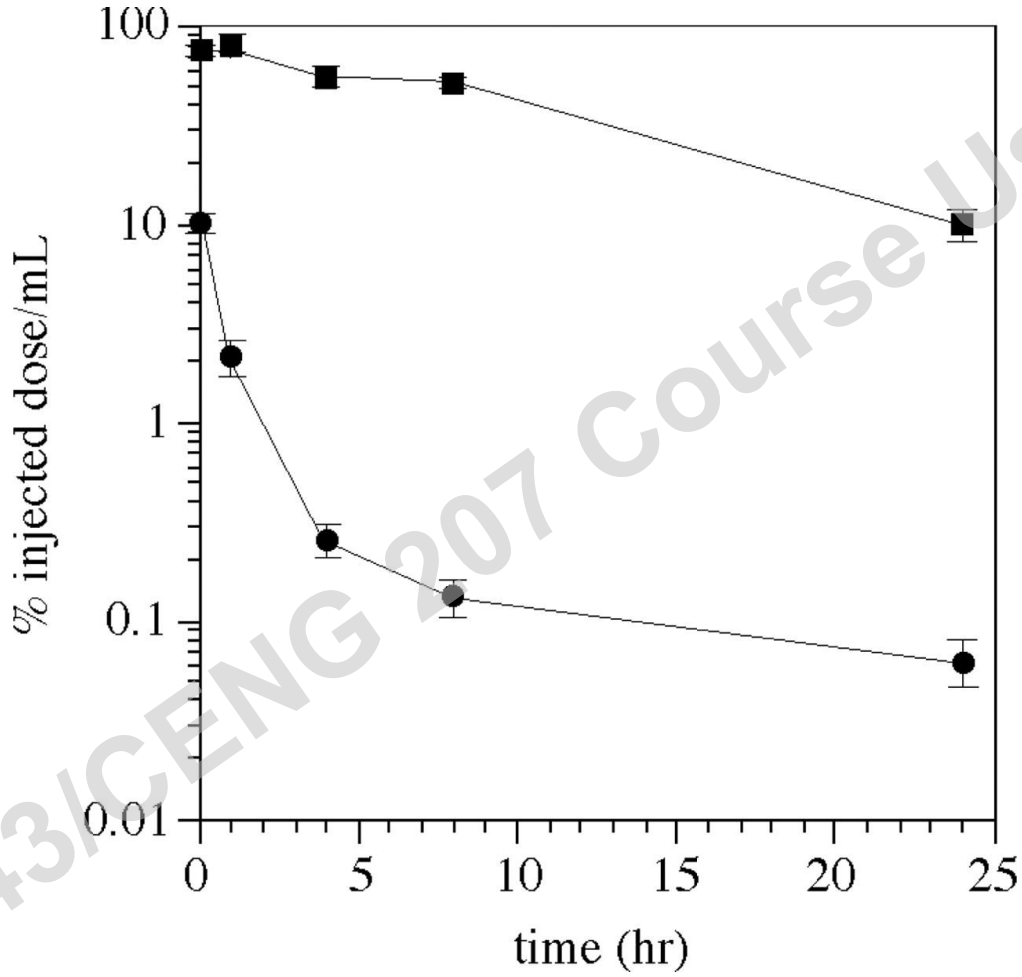
Example 3: Cisplatin-Polymeric Micelles



Chemical structures of CDDP (A) and PEG-P(Glu) block copolymers (B), and schematic illustrations of CDDP-incorporated micelles (C) and the hypothesized behavior of the micelles in physiological saline at 37°C (D).

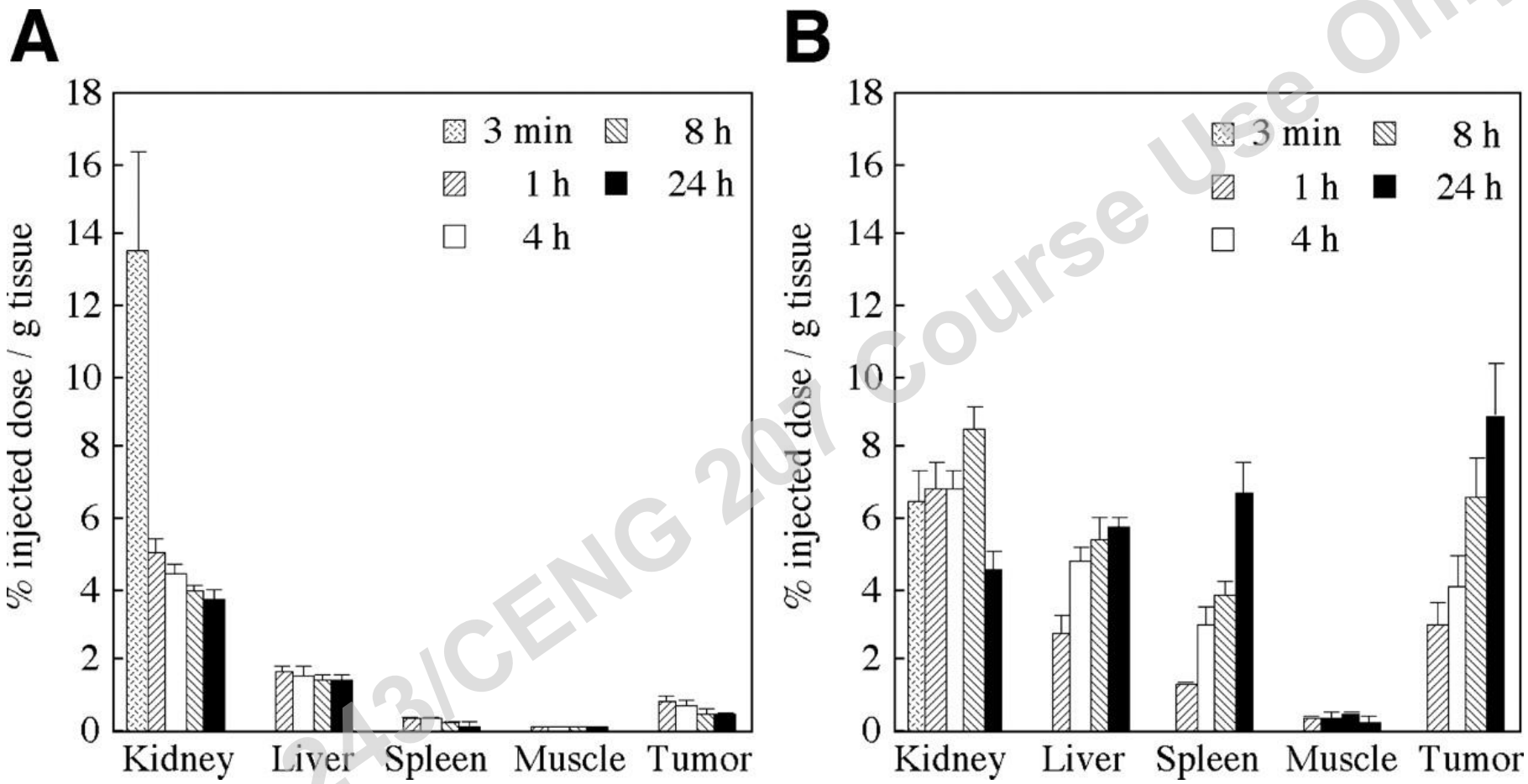
Cancer Res 2003, 63, 8977

In Vivo Pharmacokinetics



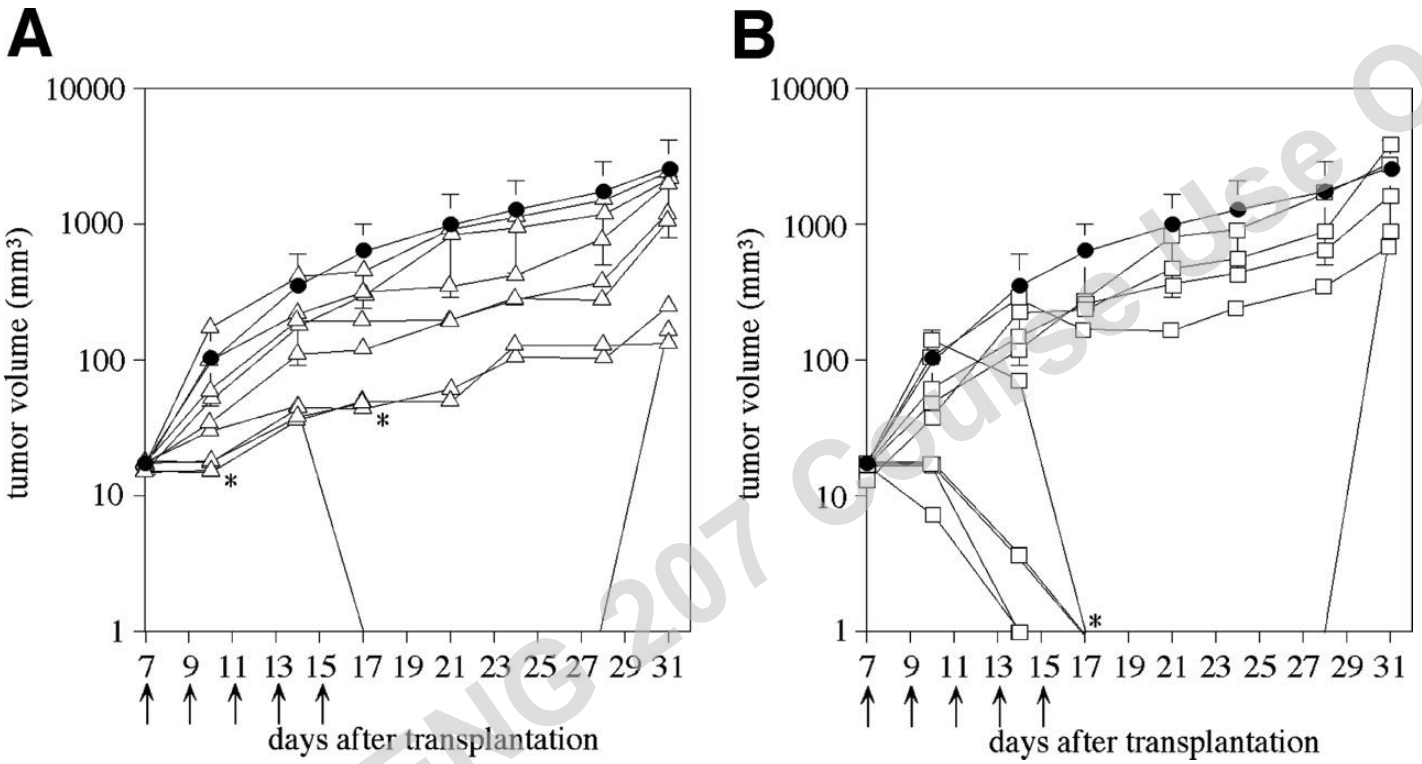
Time profiles of platinum concentration in the plasma after i.v. administration of free CDDP (•) and CDDP-incorporated micelles (▪).

Tissue Biodistribution



Tissue distribution of platinum after i.v. administration of free CDDP (A) and CDDP-incorporated micelles (B).

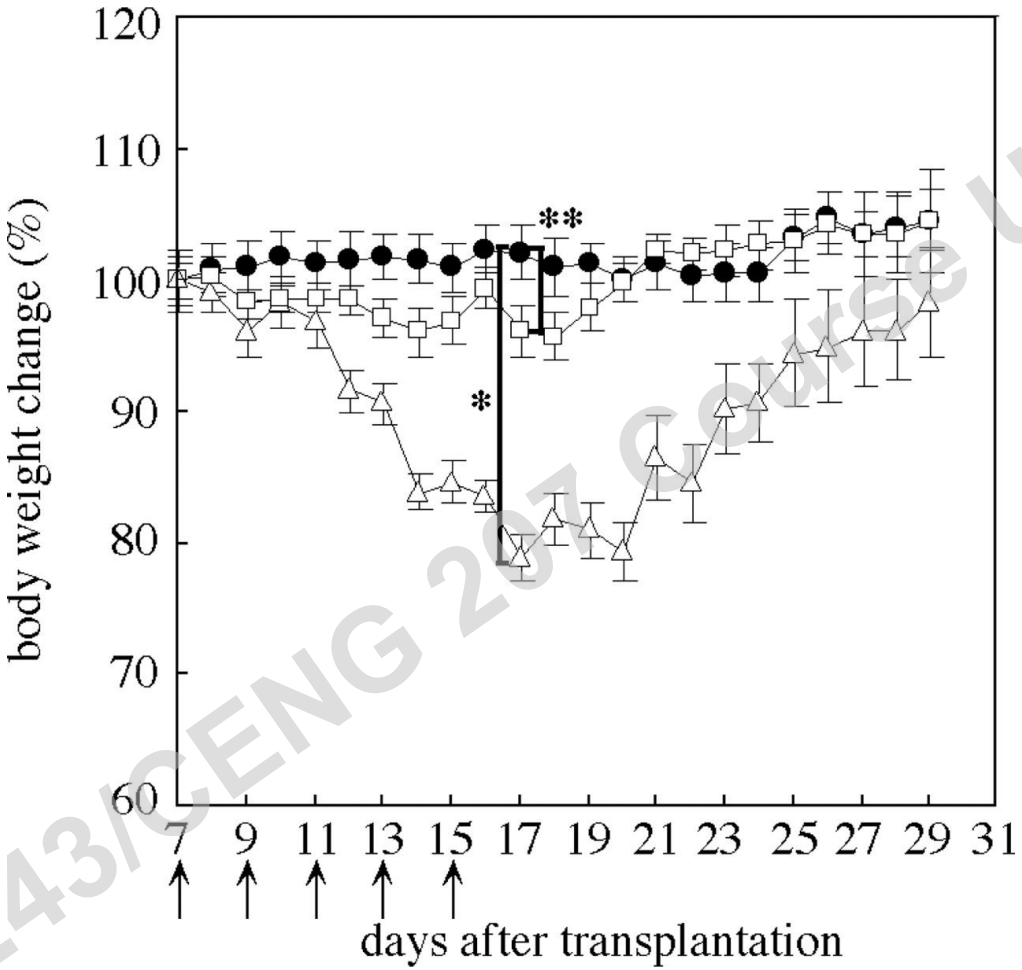
Therapeutic Efficacy



Effect of free CDDP (▯ in A) and CDDP-incorporated micelles (□ in B) on the growth of C 26 colon adenocarcinoma s.c. transplanted to CDF1 mice (female, n = 10).

Results: 6 of 10 mice treated with CDDP-incorporated micelles showed complete tumor regression after the fifth administration. In those 6 mice showing tumor regression, however, 1 mouse died after day 17, and another 1 showed tumor recurrence after day 28. Consequently, 4 tumors were completely cured in the mice treated with CDDP-incorporated micelles.

Body Weight Changes



Body weight change of C 26-bearing CDF1 mice (female, n = 10) treated with saline (•), free CDDP (□), and CDDP-incorporated micelles (□).

Summary

With the correct combination of a targeting ligand, an appropriate nanoparticle platform amenable to scale-up, a suitable drug/imaging agent, and a carefully selected disease indication, self-assembled targeted nanoparticles can be developed for safer and more effective therapeutic or imaging applications.

More complex targeted nanoparticle systems, which combine imaging and therapeutic agents or can trigger drug release at the target site when exposed to external stimuli (e.g., pH, ionic strength, enzyme, redox potential, temperature, light, ultrasound, magnetic field, and electric current), are also subject of ongoing research.

The “magic bullet” vision of Paul Ehrlich over 100 years ago is beginning to be realized, and with continued research and development efforts targeted nanoparticles are expected to have a tremendous impact on human health for decades to come.

Acc. Chem. Res. 2011, *44*, 1123