„The Magic Bullet“

Nobel in Medicine - 1908

Paul Ehlrich vision

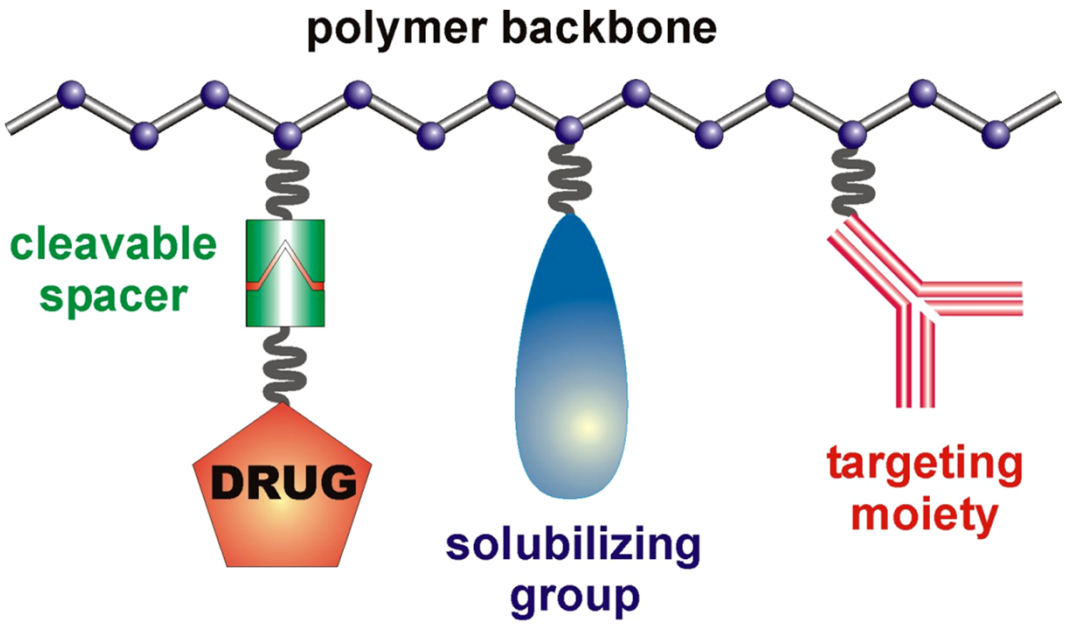
(begining of 20th century)

„The magic bullet“

mAbs as a carriers of drugs

Ringsdorf Model

Ringsdorf Model (1975)



Chemistry of bioconjugation

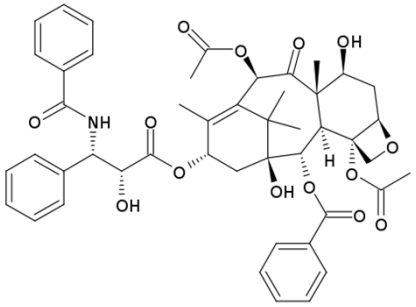
Eg. PK2, CT-2130 etc.

Drug Classes & Modes of Action

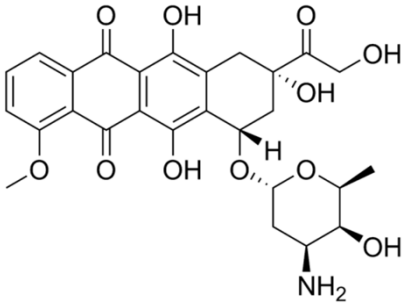
Principles of chemotherapeutic treatment

- Targeting the tumor cell
  ➔ **cytostatic drug**

  Inhibition of cell growth and proliferation



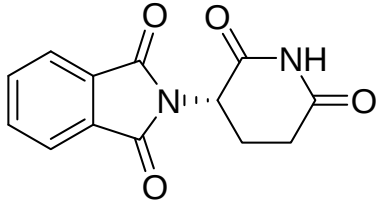
Paclitaxel



Doxorubicine

- Targeting the blood supply of the tumor
  ➔ **anti-angiogenetic drug**

  Inhibition of angiogenesis (the growth of new blood vessels)

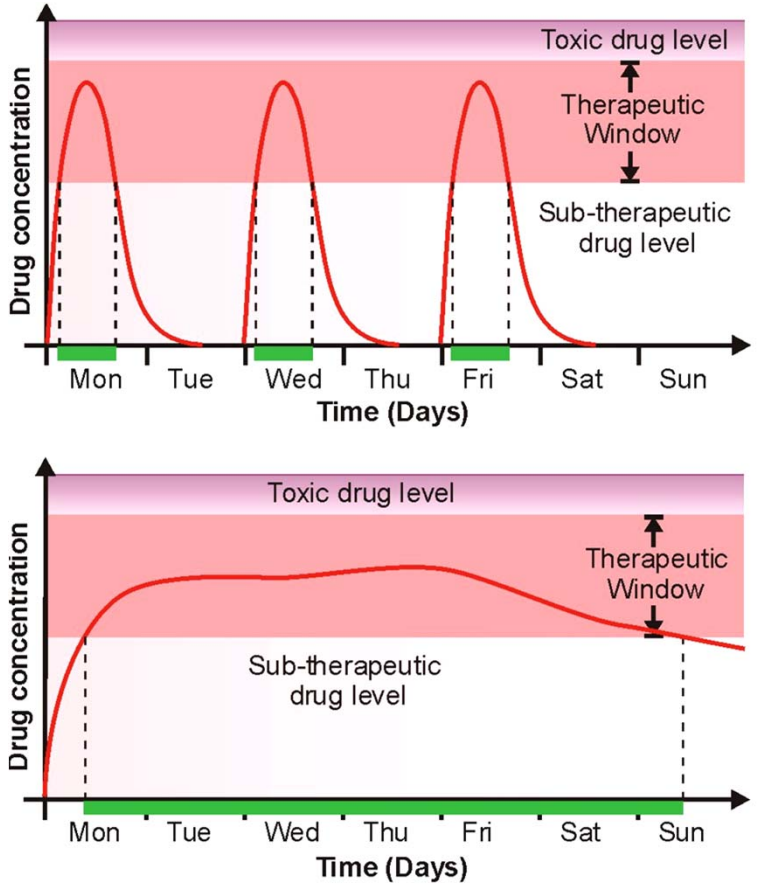


(S)-Thalidomide

Low Molecular Weight Drugs

- most drugs are small molecules (MW ~ 500 g/mol)
- short half life in blood circulation
- fast diffusion into healthy regions
- fast clearance from body
- low selectivity for target tissue causing side effects
- low amounts of drug reach target tissue
- no constant levels of drug concentration
- many side effects
- multiresistance
- poor selectivity
- poor water solubility
- high toxicity

Circulation life-time difference between unmodified drug and polymer-drug conjugate



Pharmacokinetic profile of Interferon and **Pegasys®** - Interferon conjugated to branched PEG 40 kDa

Low Molecular Therapeutics vs. Macromolecular Therapeutics

Low Molecular Therapeutics		Macromolecular Therapeutics
▪ Slubility problems	⟺	▪ Improved water slubility
▪ Cell entry by diffision	⟺	▪ Cell entry by endocytosis (abbility to overcome multidrug resistance)
▪ No preferential accumulation	⟺	▪ Preferentia accumulation in solid tumors
▪ Targeting (prodrug) possible	⟺	▪ Effective targeting - cell surface receptors - specific subcellular organelles
▪ Fast elimination	⟺	▪ Slower elimination
▪ Non-specific toxicity	⟺	▪ Decreased non-specific toxicity

Polymers in Drug Delivery, Taylor, 2006, Uchegbu, p 159.

Requirements for Polymeric Drug Carriers

Polymeric drug carriers have to be:

1. Well characterized (reproducibility, PDI, heavy metal traces)
2. Hydrophilic for intravenous drug delivery.
3. Biocompatible (non-toxic and non-immunogenic), also metabolic products
4. Multivalent (for conjugation of drug, targeting and/or imaging moiety)
5. Stable in circulation (drug linkage)
6. Formulation (stabile under convenient administration)
7. Easly eliminated from the body (whole polymer or metabolites)

Polymers in Drug Delivery, Taylor, **2006**, Uchegbu, p 161.

Cancer Therapy

Solution: **Tumor specific delivery**

Higher Selectivity to Cancer Cells

Passive Targeting

Based on Tumor Vasculature

specifiic phisiological differences:

True for tumors bigger than **2-3 mm**

Active Targeting

Based on Tumor Cell-Surface Receptor

- Antigene
- Growth Factors
- Peptide receptors
- Carbohydrate receptors
- Vaccins receptors

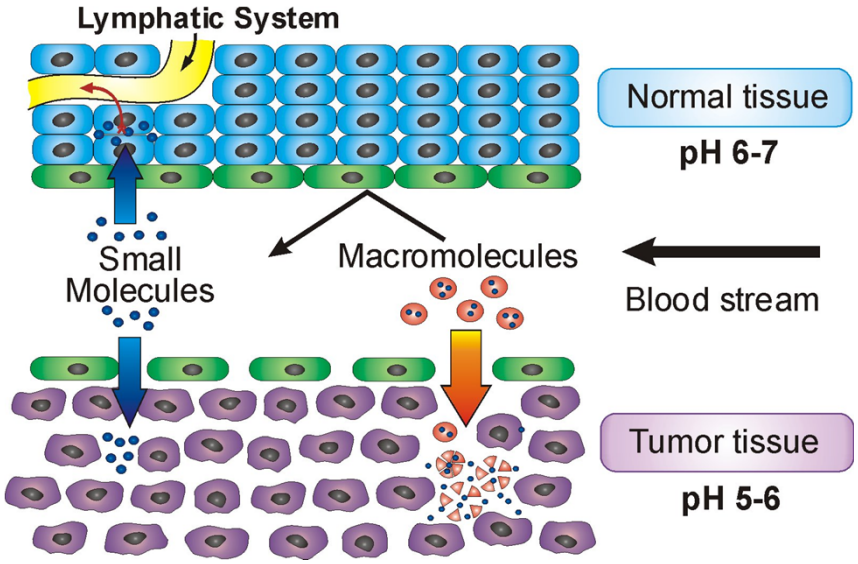
Passive Targeting

Selectivity → Passive targeting

Enahnced **P**ermeability and **R**etention **E**ffect (***Maeda***, 1986, SMANCS)

- Intravenous administration of macromolecular therapeutics
- Size-dependent selectivity of drugs entry

Tumor size: **2-3 mm** ⇒ cell clusters inducing angiogenesis
Pore size: **100 – 1200 nm** (depending on tumor type)

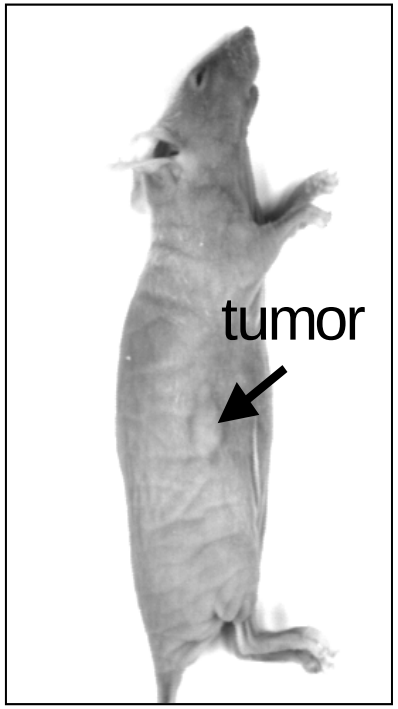


<u>Structural features of macormolecules:</u>

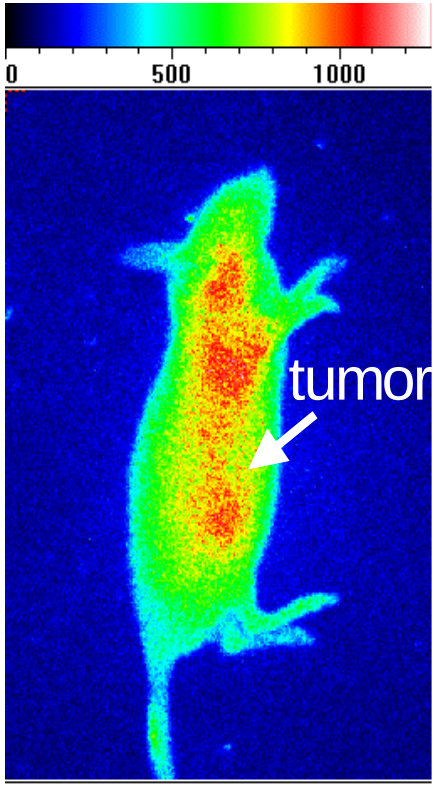
⇒ molecular weight (> 40kDa)
⇒ charge (negative or neutral)
⇒ shape (globular, linear, branched)
⇒ hydrodynamic radius

Uncharged or negatively charged macromolecular carriers that larger than 40 kDa efficiently escape renal clearance.

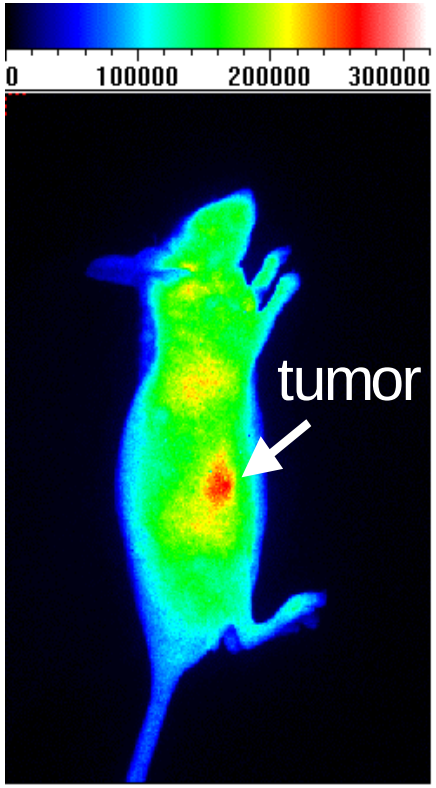
Demonstration of the EPR-Effect



K. Licha, F. Kratz



Indotricarbocyanin
(unbound dye)
after 24 h

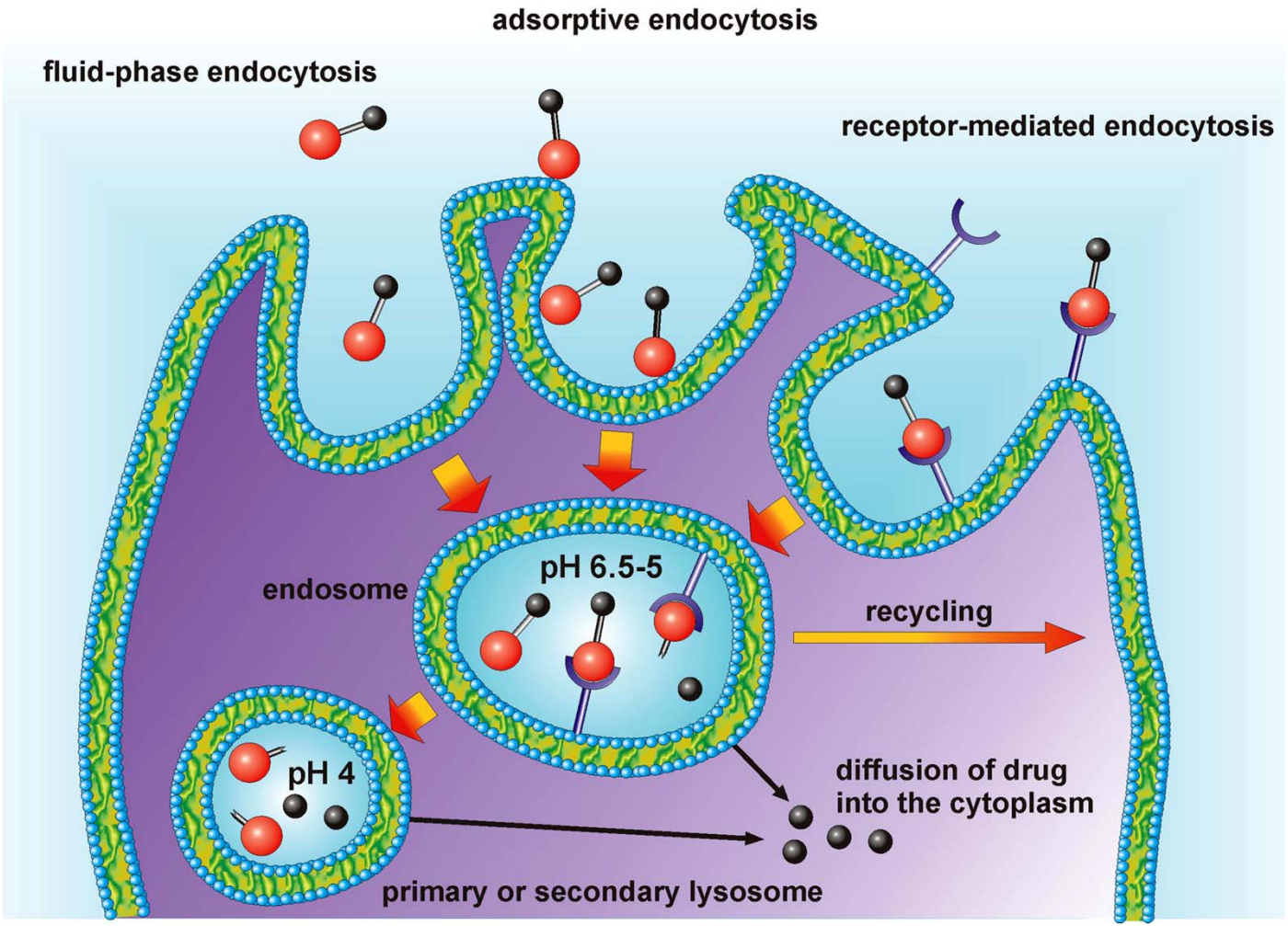


PEG 40kDa-Indocarbo-cyanin-conjugate
after 24 h

Problem: Tumor specificity only with nanoparticles >5 nm

Active Targeting

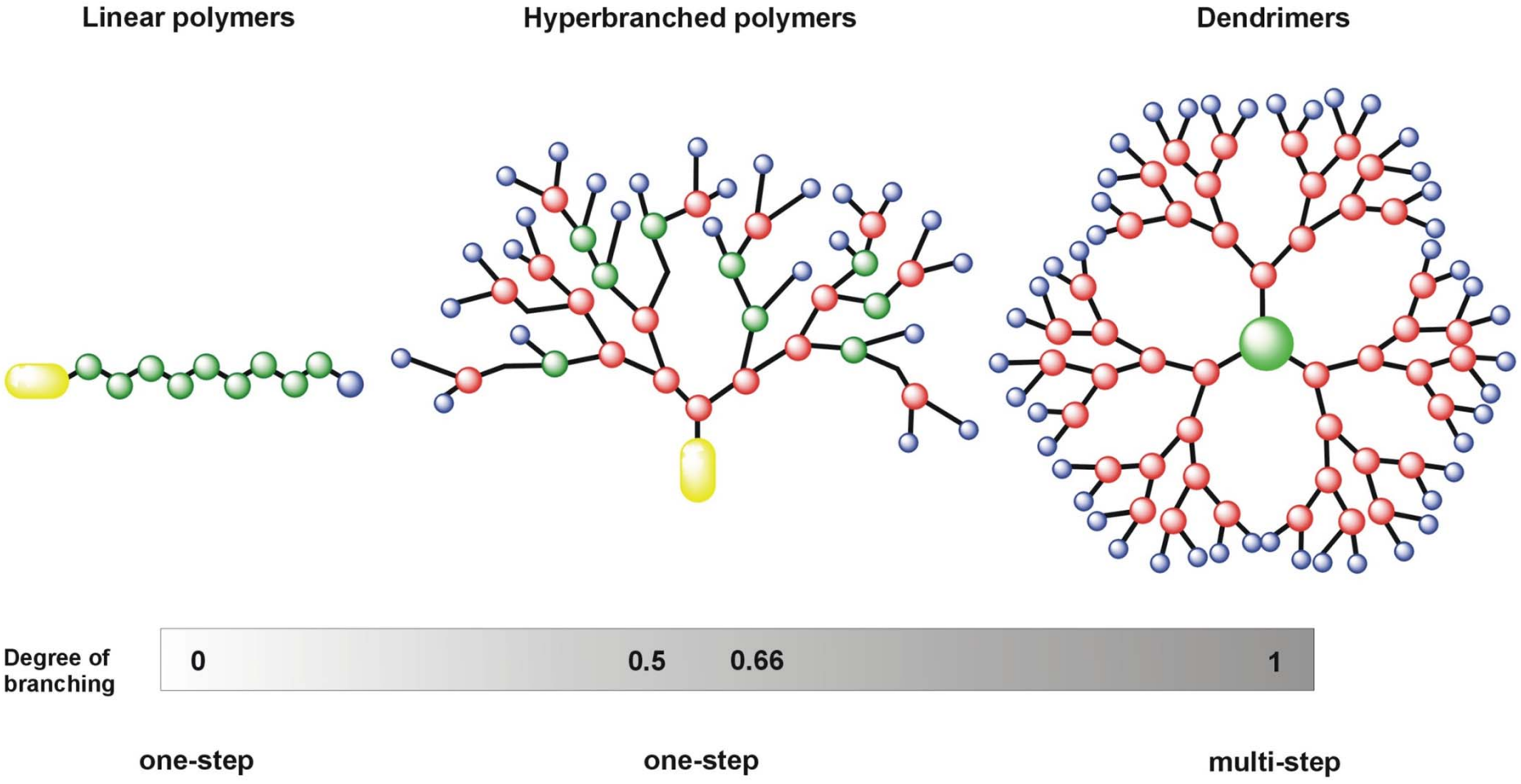
Selectivity → Active targeting



Endocytotic pathway for the cellular uptake of macromolecules and nanocarriers for drug delivery

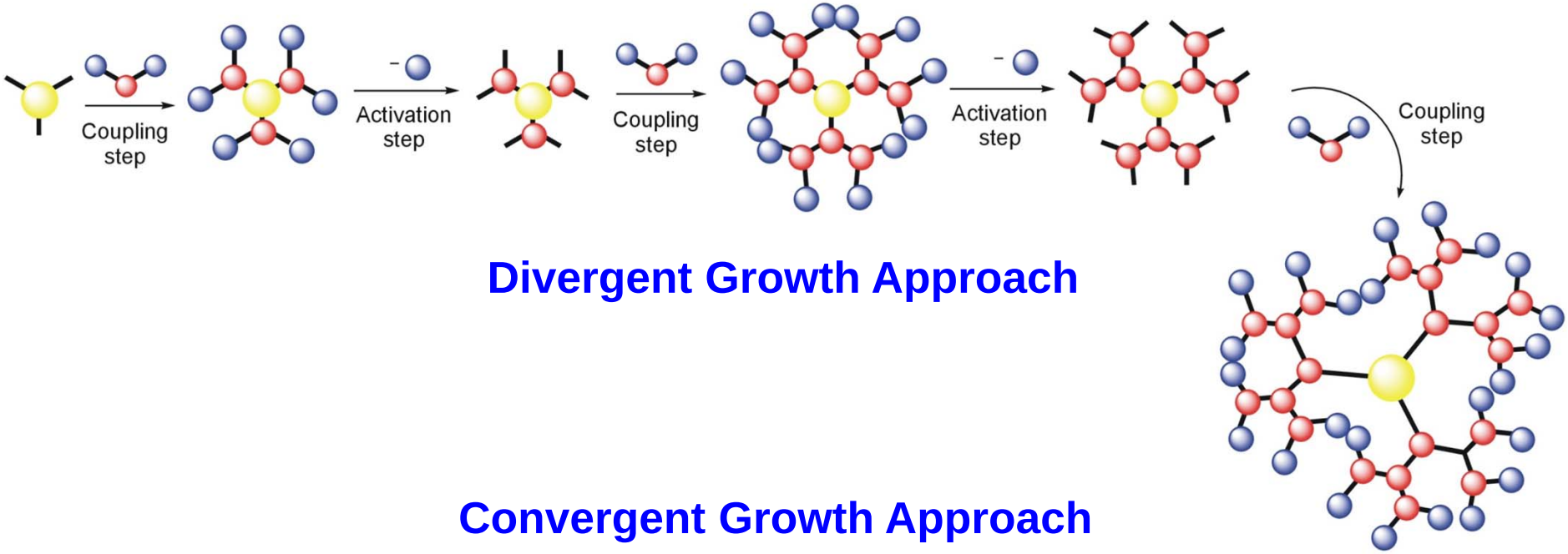
Dendritic Polymers in Biomedical Applications - From Synthesis to Clinical Use

Degree of Branching



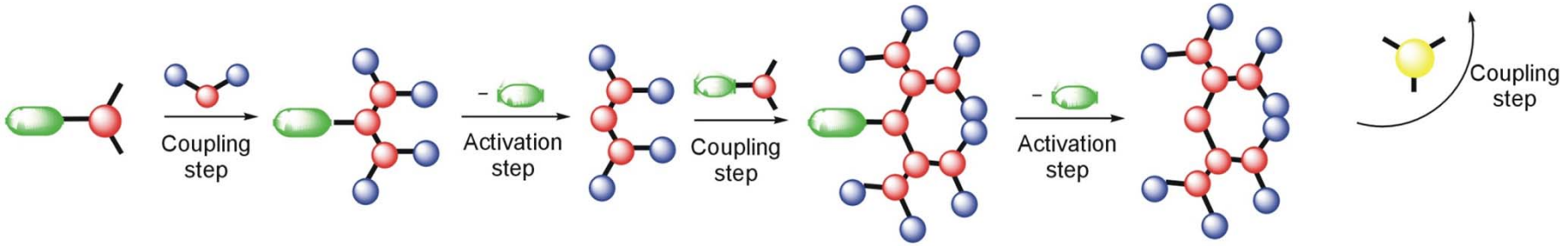
Synthesis of Dendrimers

from core to the shell



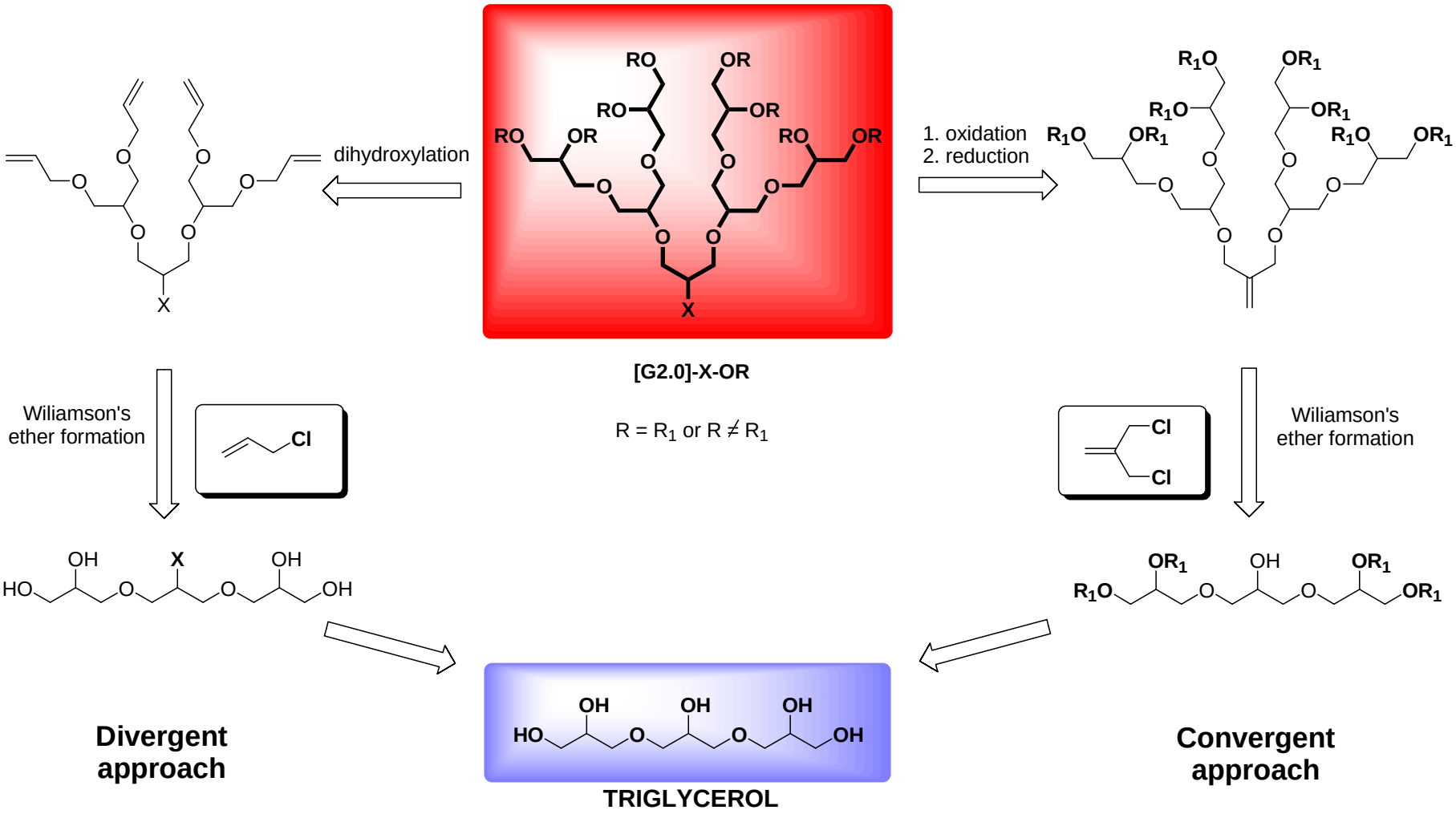
Divergent Growth Approach

Convergent Growth Approach



from shell to the core

Synthesis of Polyglycerol Dendrons



Properties of Dendrimers

Advantages:

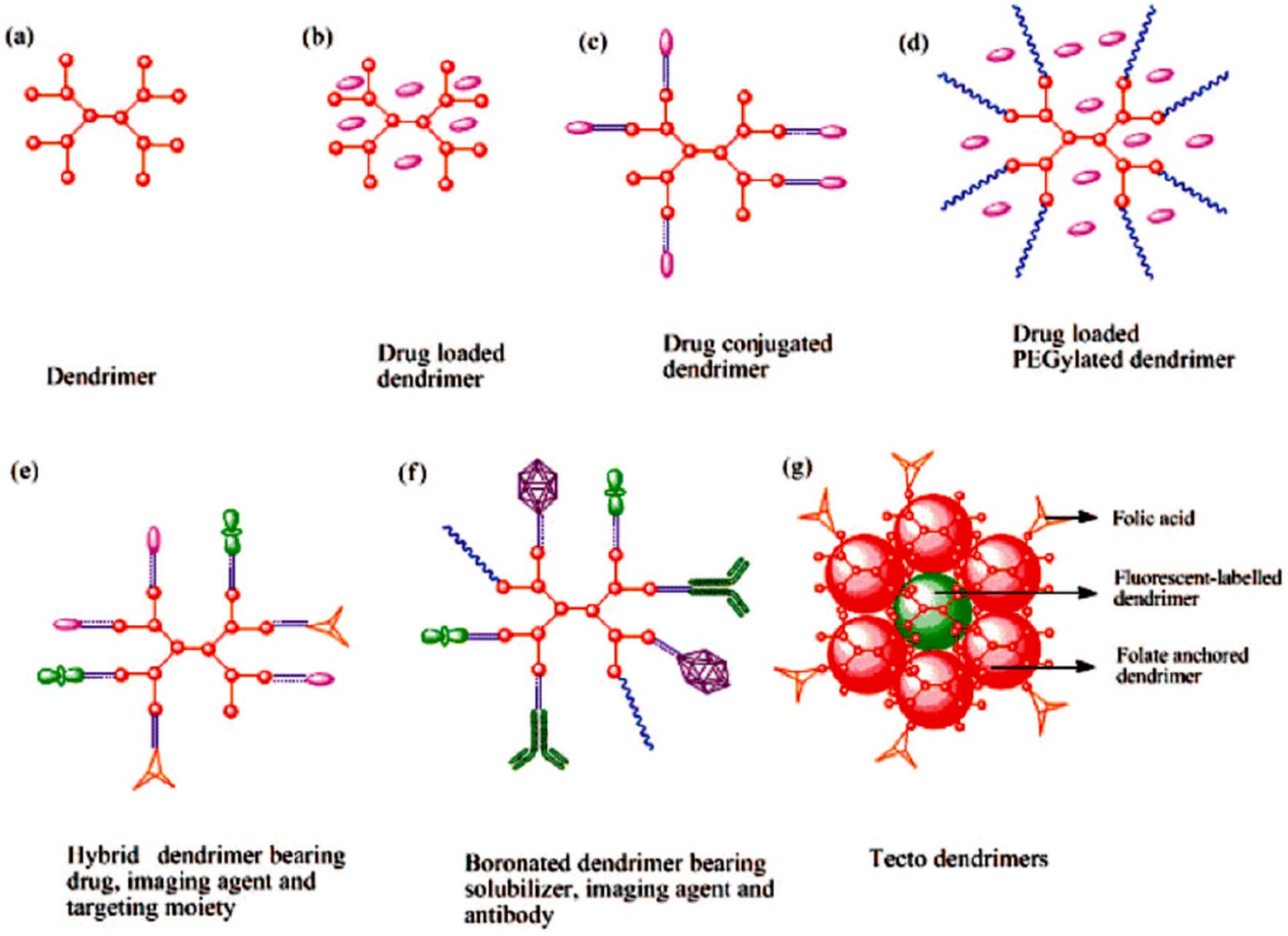
- highly branched
- highly reactive
- three-dimentional
- high structural purity (low PDI)
- monodispercity (single MW)
- globular shape
- large number of „peripheral“ functionalities
- bifunctionality
- multivalency

Disadvantages:

- step-by-step synthesis (tedious)
- high costs of production
- „mistakes“ in the structure

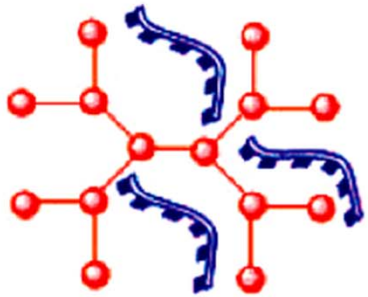
A. W. Bosman, H. M. Janssen, E. W. Meijer *Chem. Rev.* **1999**, *99*, 1665-1688.

Dendrimers in Cancer Therapy and Diagnostic



Dendrimers in Cancer Therapy and Diagnostic

(h)

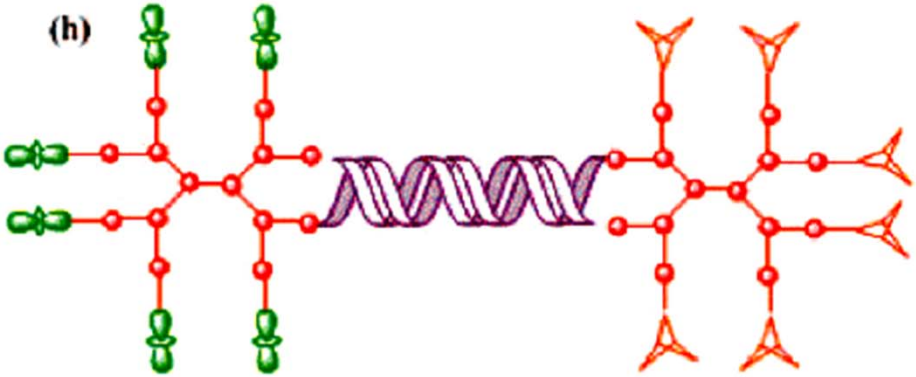


Dendrimer-oligonucleotide complex

(i)

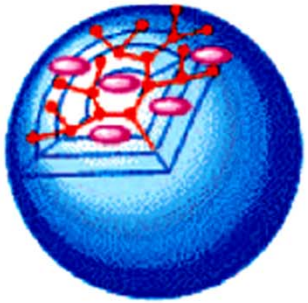
Dendrimer-DNA complex

(h)

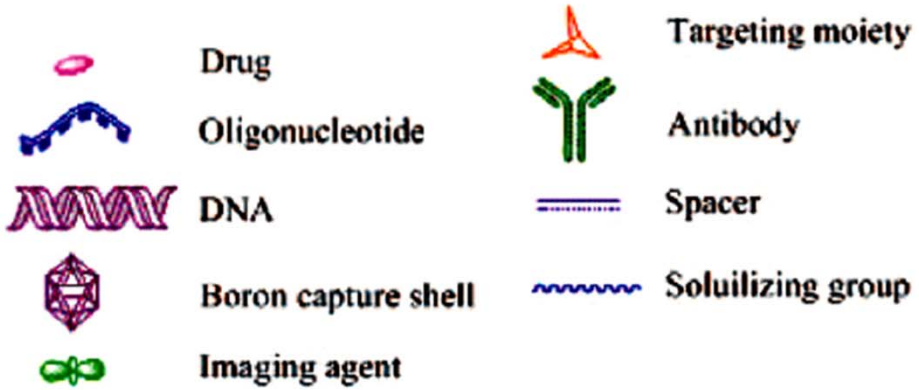


DNA assembled Hybrid dendrimer

(k)



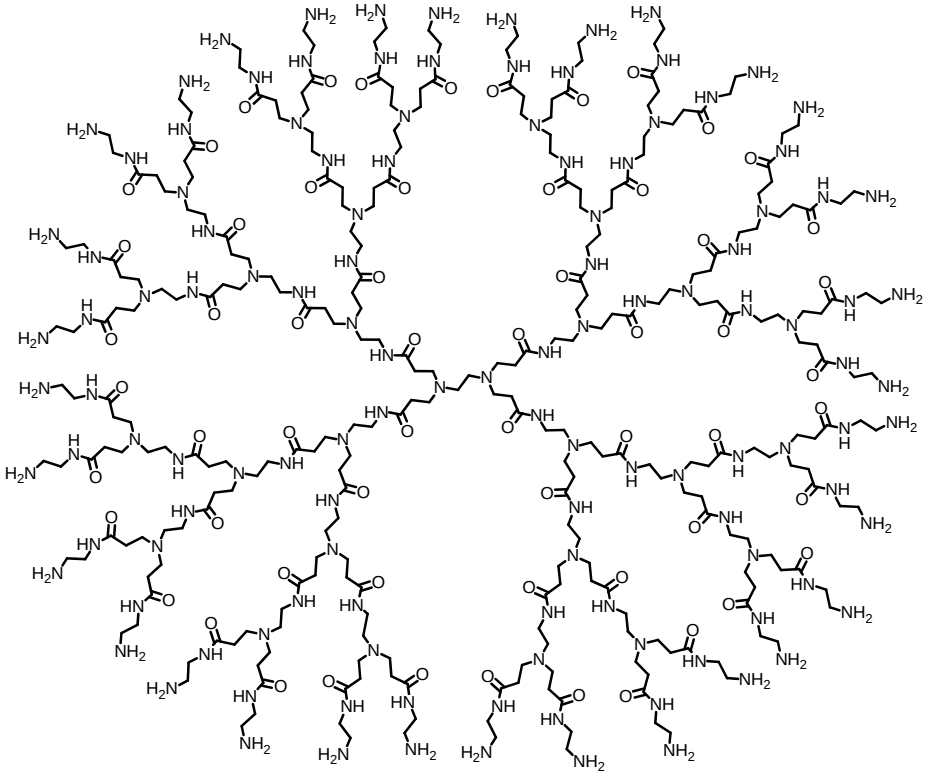
Drug loaded liposomal "locked in" dendrimers



First Dendrimers

[G4.0] PAMAM - Poly(amido amine)

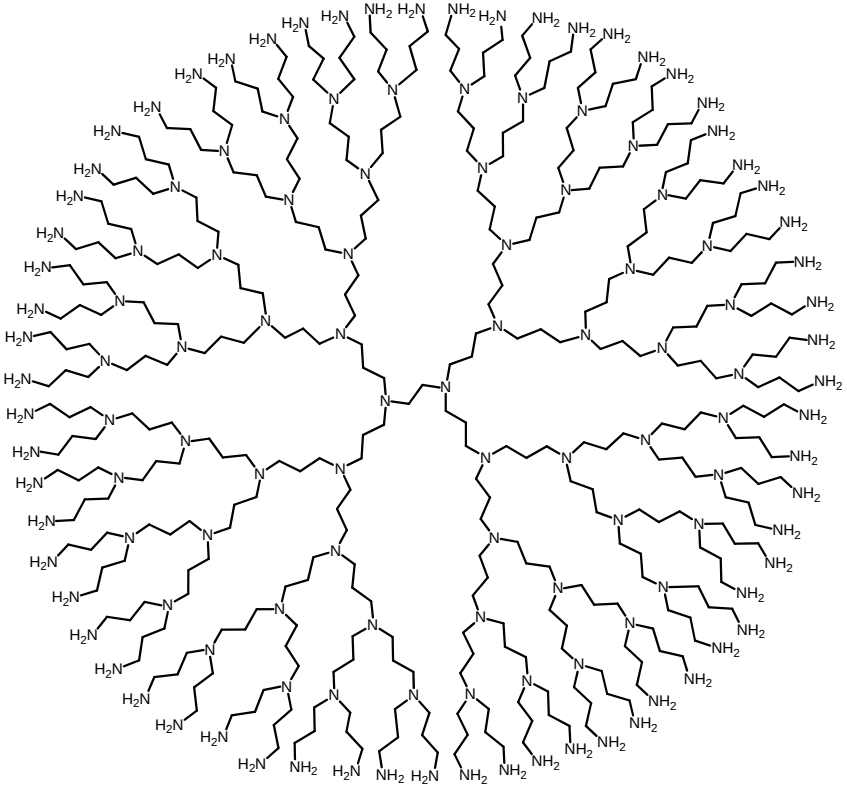
1985 – Don Tomalia



Starburst® (Dendritech)

[G5.0] PPI* - Poly(propylene imine)

1978 – *F. Vögtle*
1993 – *E. W. ´Bert´ Meijer*
and indepedently ***G. Mülthaupt***

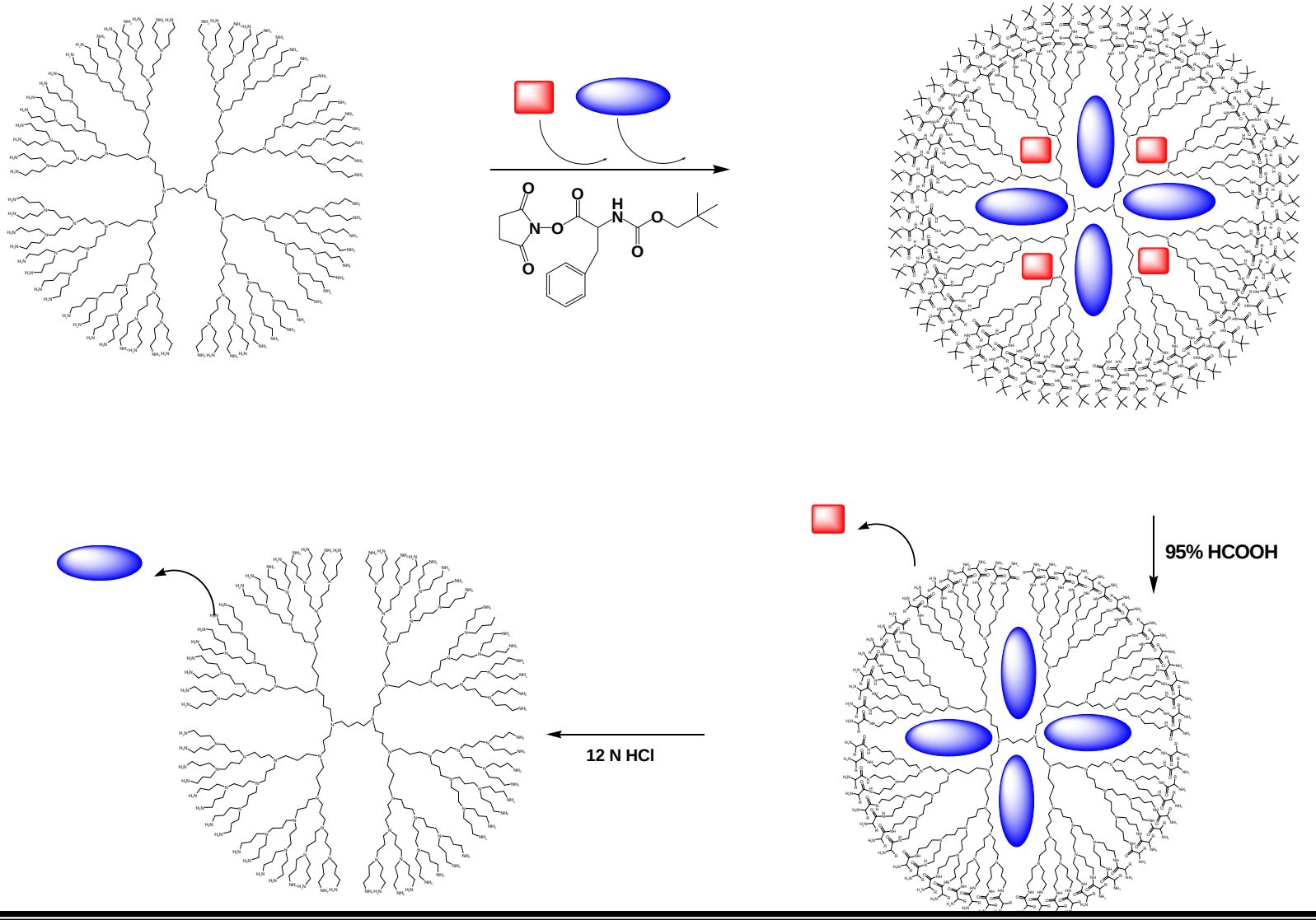


Astramol® (DSM Fine Chemicals)

* known also as **POPAM**, or **DAB** with **Di**ami**no**Butan

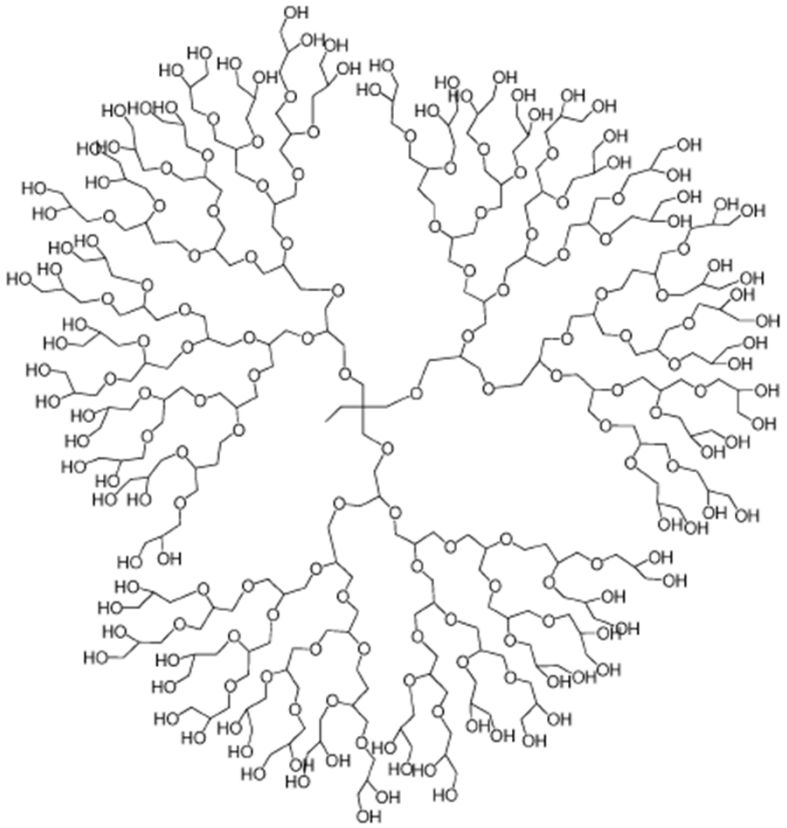
Drug-Loaded Dendrimers

Shape-Selective Release of Guest



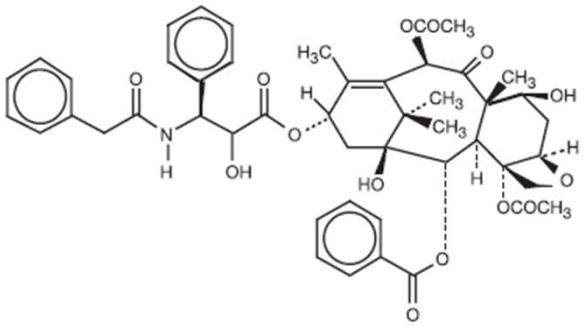
Drug-Loaded Dendrimers

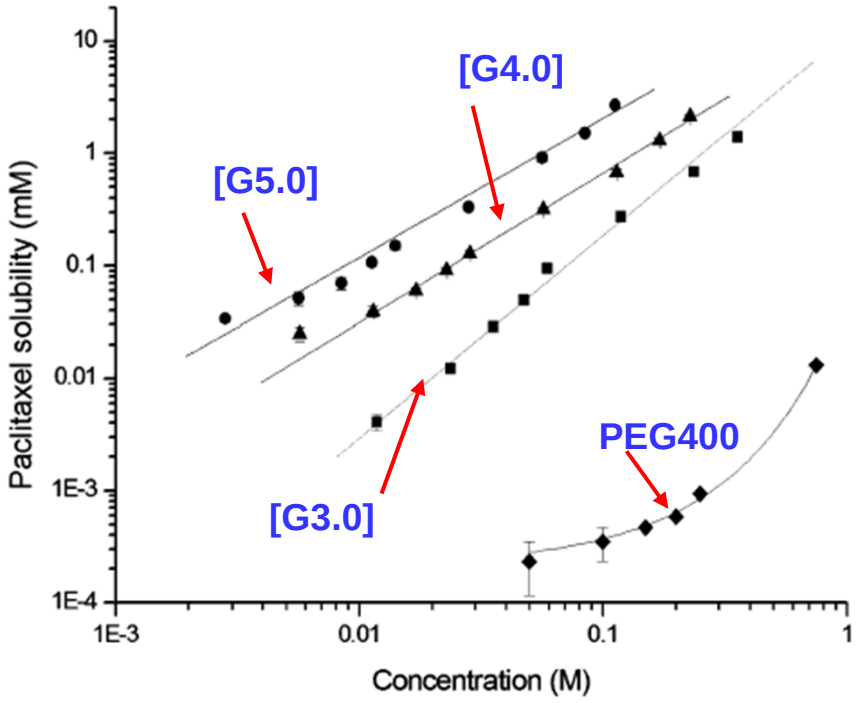
Solubilization of Paclitaxel by PG Dendrimers



PGD G-5

PTX

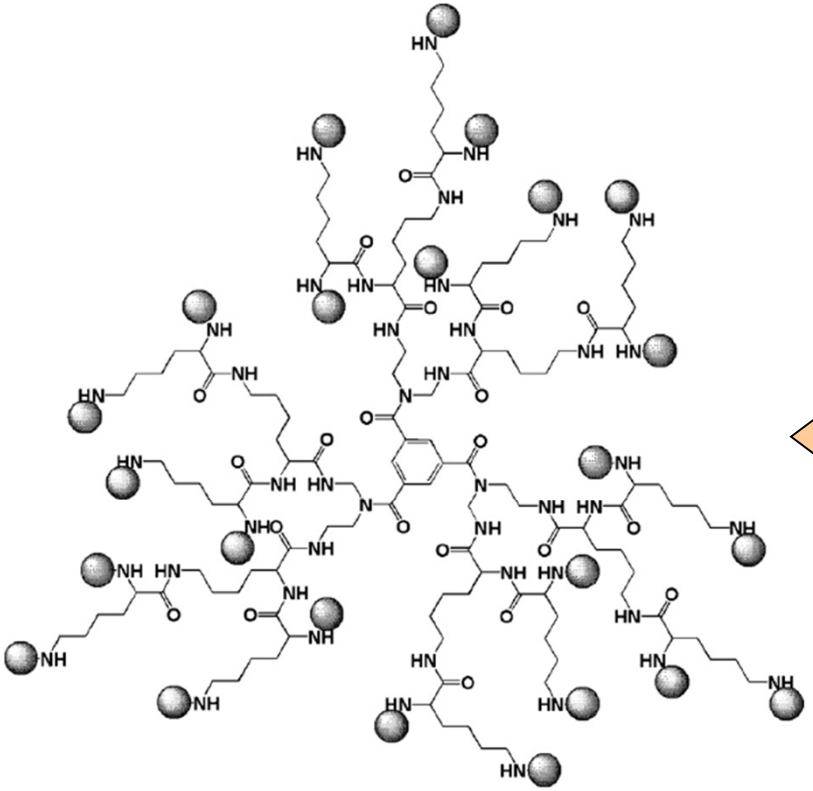




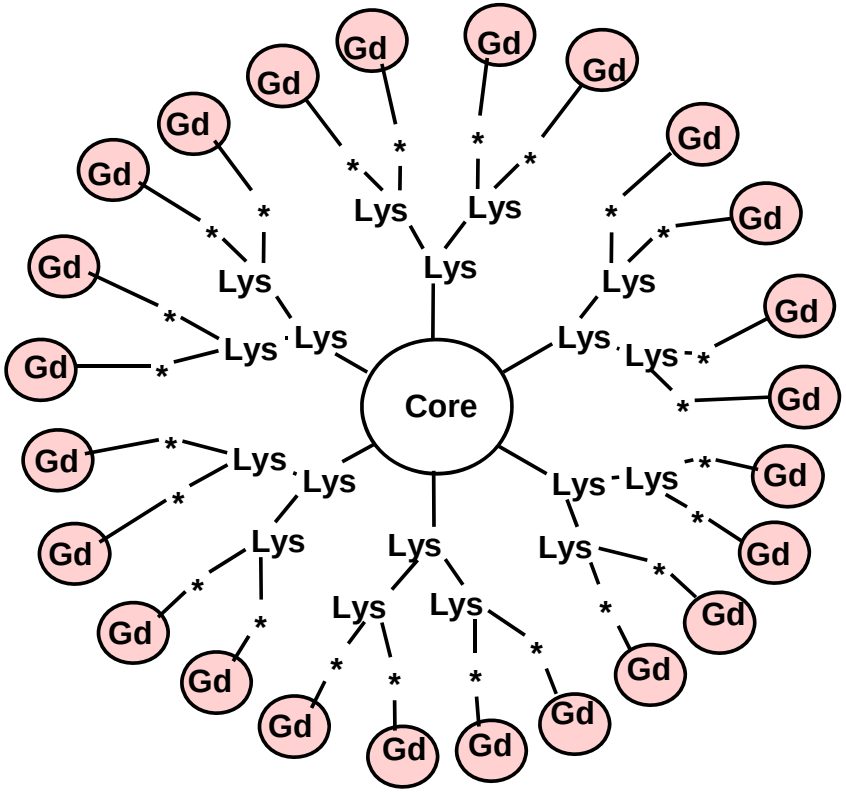
10.000 fold better solubilization than in H_2O

Dendrimers for MRI

Gadomer®-17 (Schering AG)



DTPA

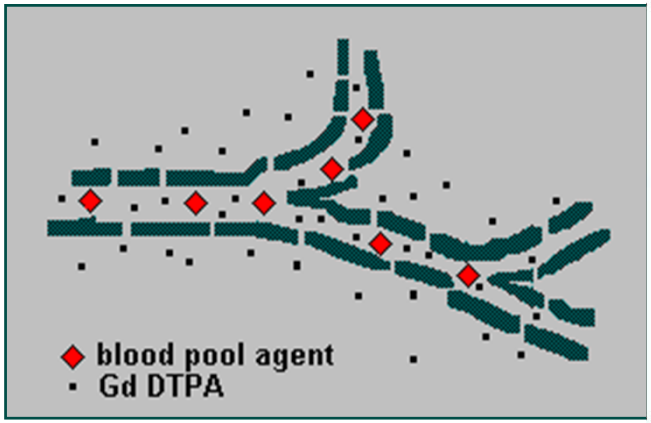


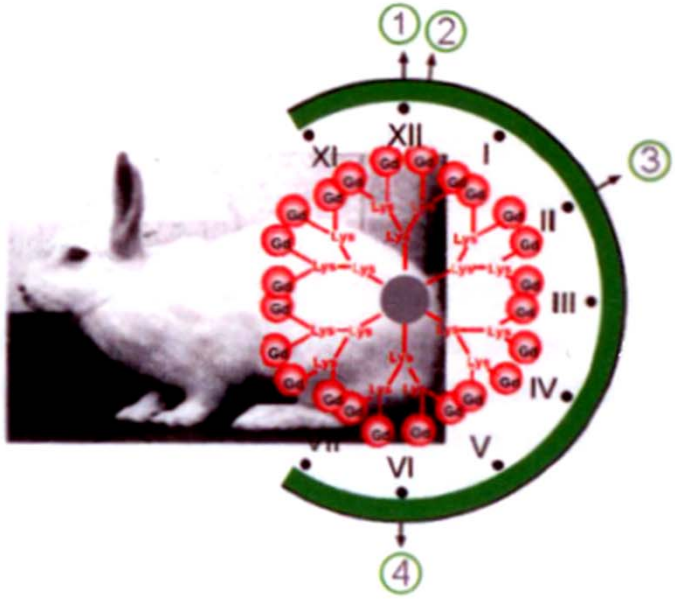
(Bio)Polymers in Biomedical Applications - From Lab to Clinical Use

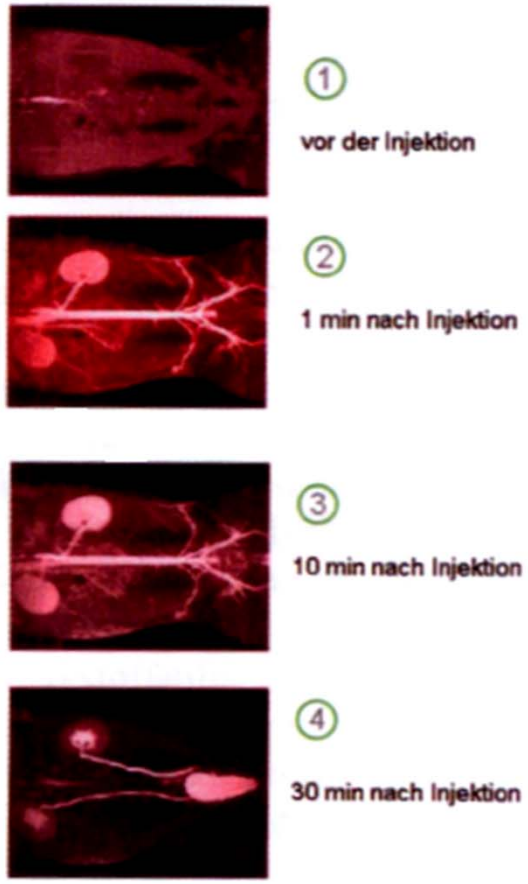
Dendrimers for MRI

Gadomer®-17 (Gadomer-24)

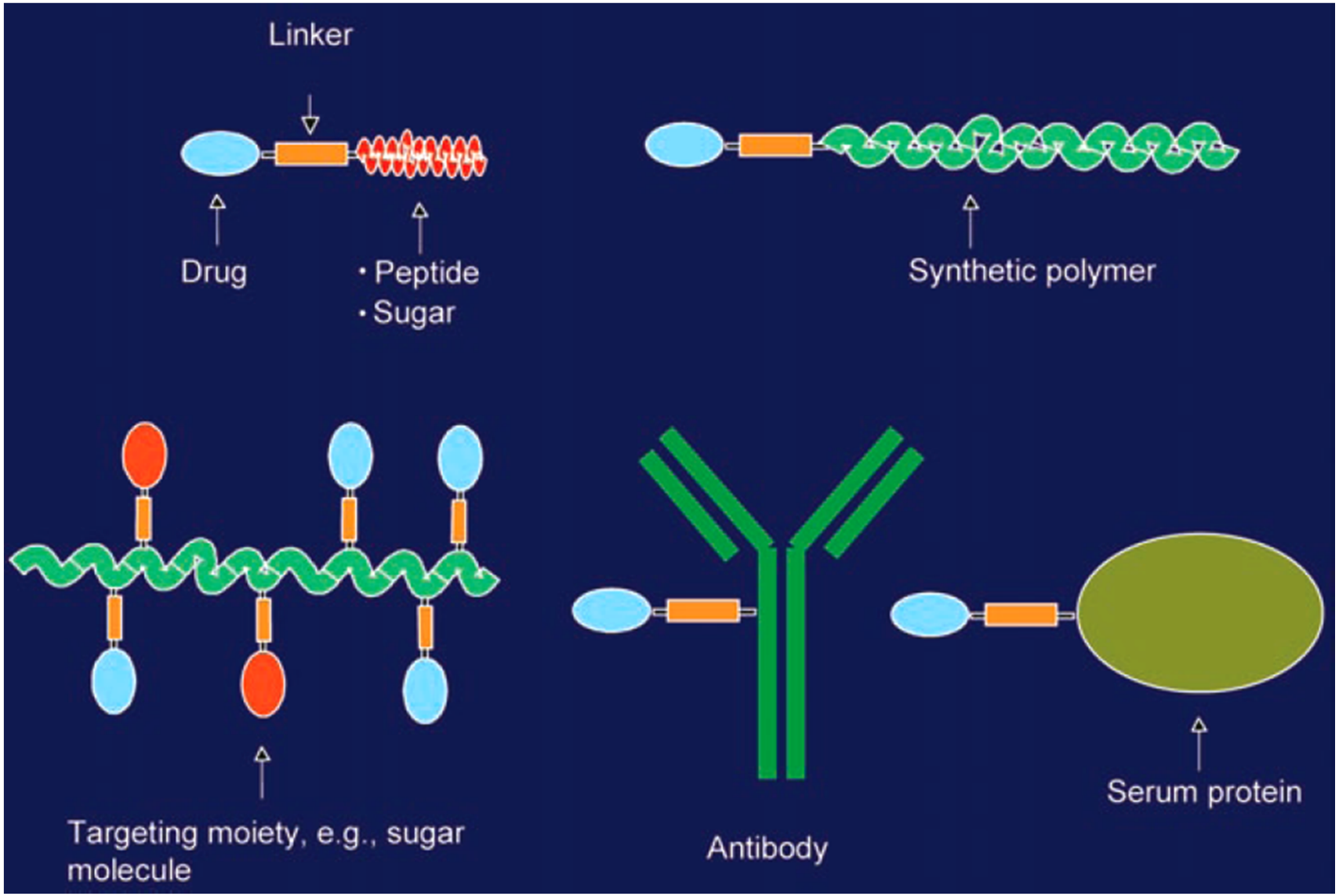
- Polylysine dendrimer carrying 24 Gadolium complexes on the surface
- MW = 17 kDa
- blood pool contrast agent
- high in vivo stability





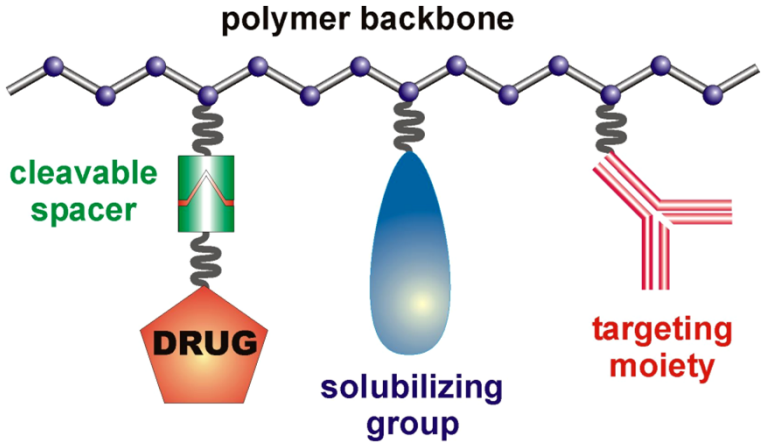


Polymer-Drug Conjugates

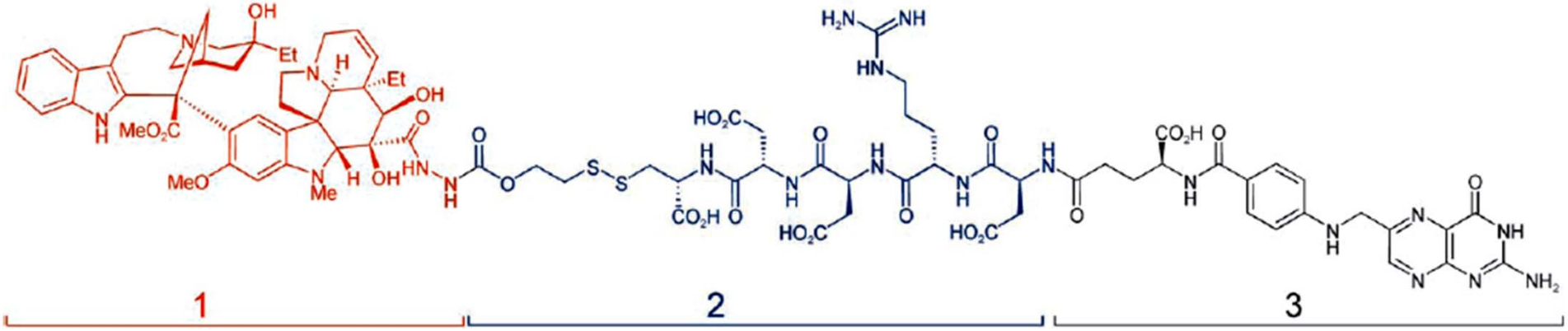


Folate-Targeted Cancer Therapeutic

EC145 (Leamon, 2006)



- Vinca alkaloid desacetylvinblastine monohydrazide (DAVLBH, a depolymerization inhibitor)
- Water soluble spacer - arginine and aspartic acid
- Release by disulfide reduction
- MTD ~ 0.8 mg/kg DAVLBH-eq.
- **Phase II clinical trial (EC145)**



Polymer-Protein Conjugate

SMANCS-Lipiodol® - approved in **1993** (Japan) for treatment of liver cancer

SMANCS -

Poly(**S**tyrene-co-**M**aleic **A**cid-half-*n*-butylate)-conjugated **N**eo**C**arzino**S**tatin

Cytotoxicity (0.01 μg/ml) against:

- mammalian cells
- gram (+) bacteria

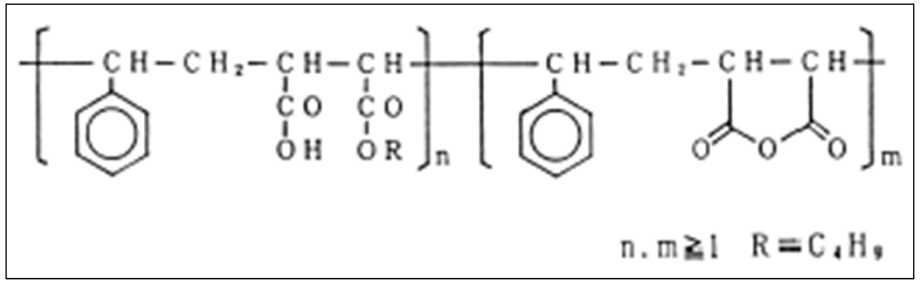
Short half-time

$t_{0.5}$ = 1.9 min

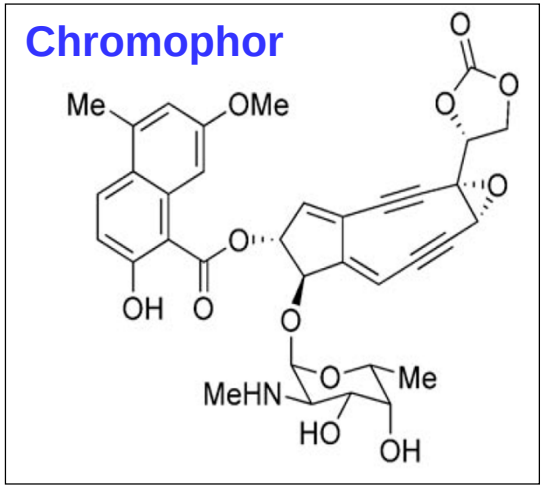
NCS - proteinaceous antitumor antibiotic (*Streptomyces carzinostaticus)*

Apoprotein (113 aminoacids) + tightly, non-covalently bound labile Chromophore

SMA – copolymer of styrene-maleic acid -half-*n*-butylate

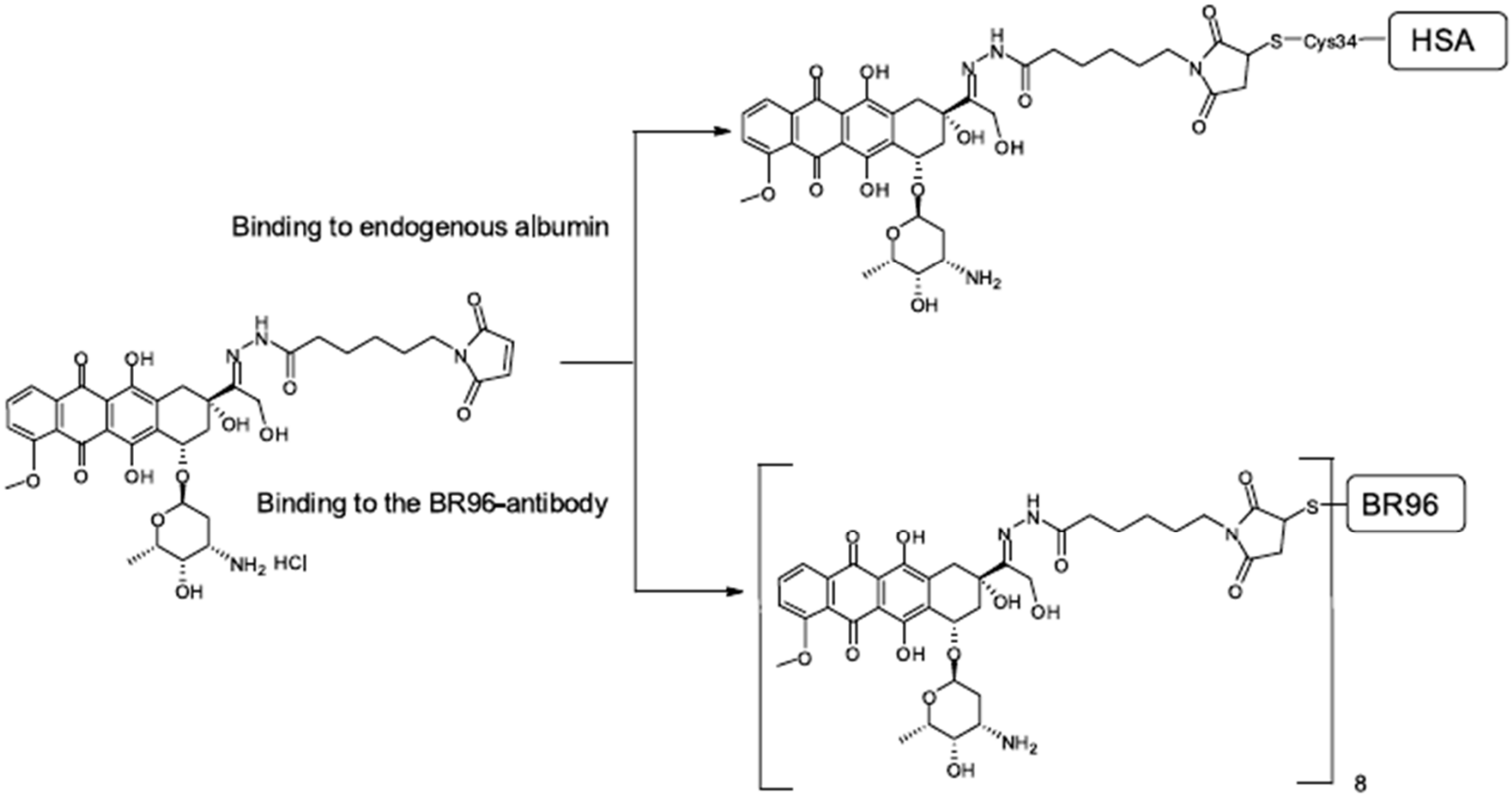


Chromophor



Biopolymer-Drug Conjugates

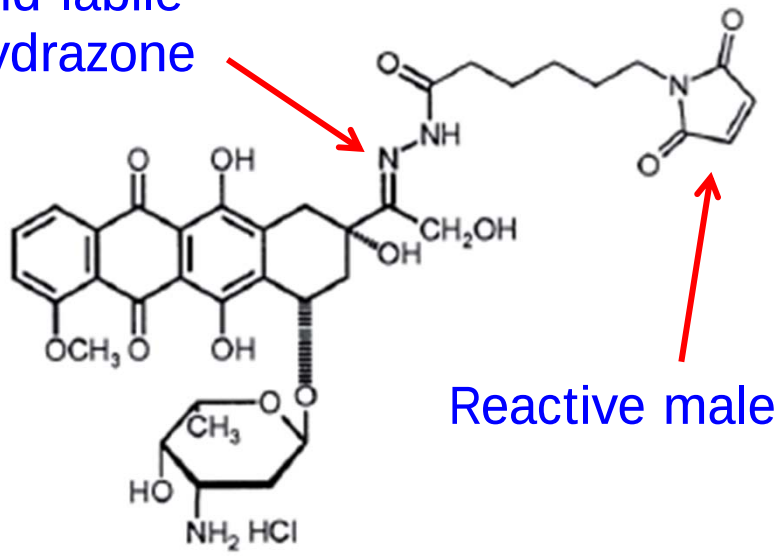
Doxo-EMCH – *in clinical studies (Phase I and II)*



Biopolymer-Drug Conjugates

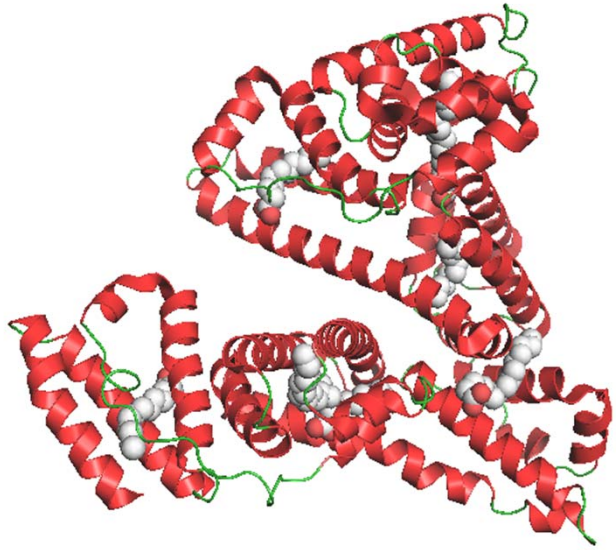
Doxo-EMCH

Acid-labile
Hydrazone



Reactive maleimid

Blood protein albumin (HSA)
as polymeric drug carrier

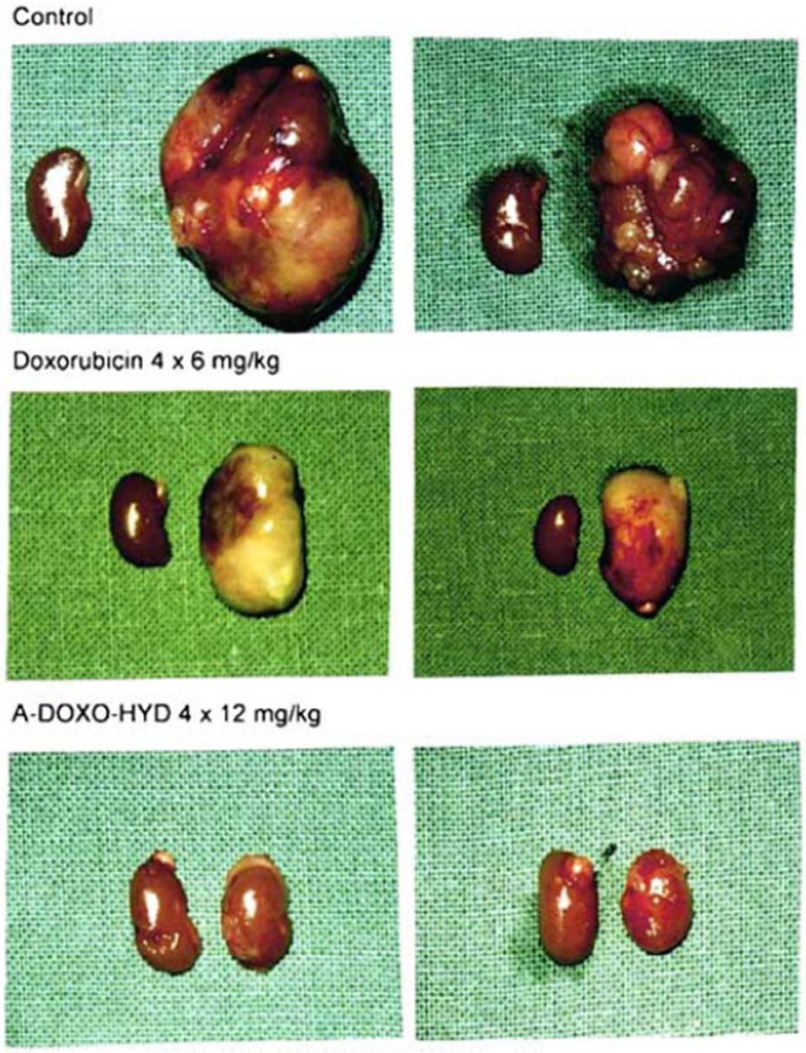


- Direct injection of Doxo-maleimid (Pro-Drug)
- Ultimate coupling to Cys-34 of HSA (70% free thiol)
- In situ generation of polymeric pro-drug
- Longer circulation, cellular uptake, higher therapeutic window

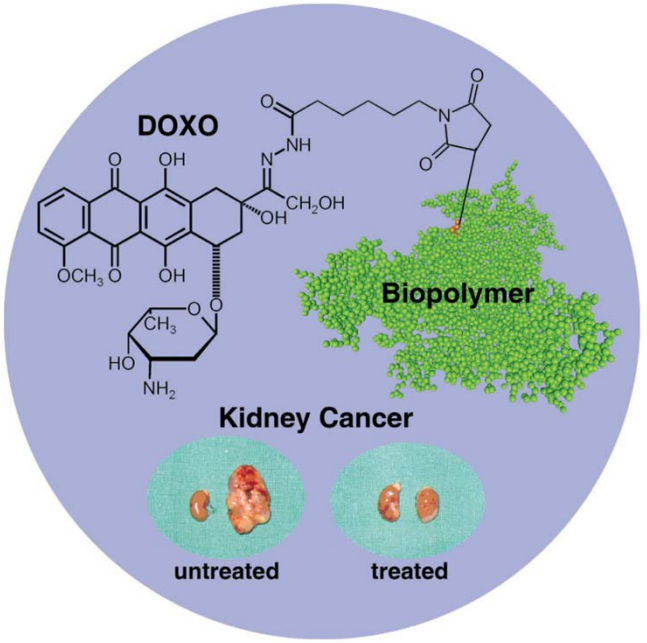
F. Kratz F, *J. Contr. Release,* **2000**, *132*, 171-183.

Biopolymer-Drug Conjugates

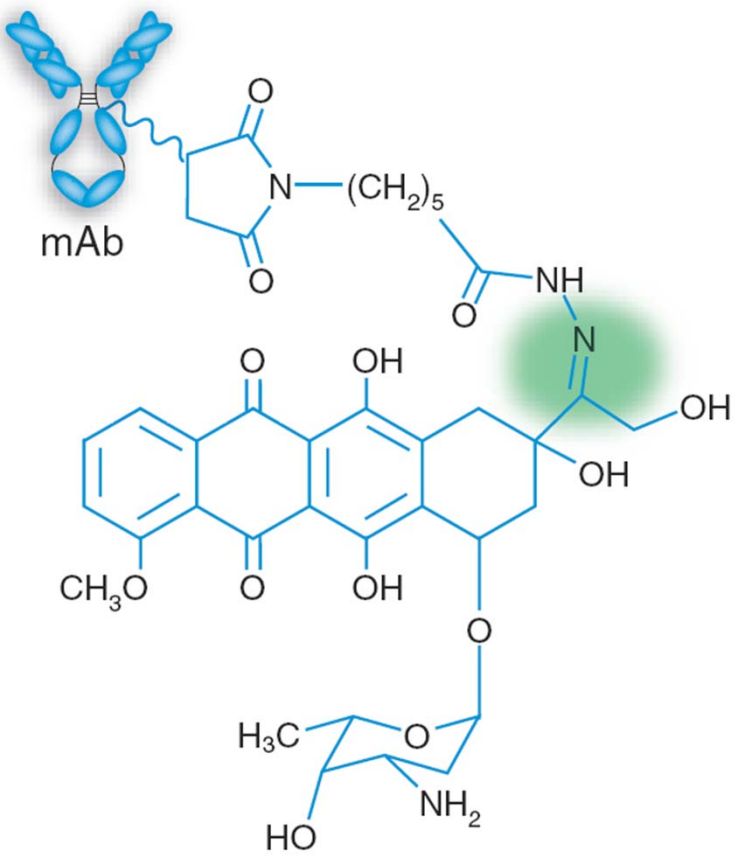
Doxo-EMCH



	Average number of lung metastases
Control	248
Albumin control	408
Doxorubicin (4 x 6 mg/kg)	94
A-DOXO-HYD (4 x 12 mg/kg)	2



Immunoconjugates



mAb-doxorubicin (hydrazone)

BR96-DOX

Structure:

- chimeric mAbs **BR96** – targeting anti-Lewis^Y
- ~ 8 DOX molecules per mAb

Phase I clinical study

66 patients, intravenous administration over 21d

Dose: 66 – 875 mg/m^2 (2-25 mg/m^2 free DOX)

Phase II clinical study

29 patients

Dose: 700 mg/m^2 (20 mg/m^2 free DOX)

- **cross-reactivity** of BR96-DOX with normal gastrointestinal tissue
- BR96-DOX demonstrated **synergistic effects** with paclitaxel and docetaxel (Taxotere®)

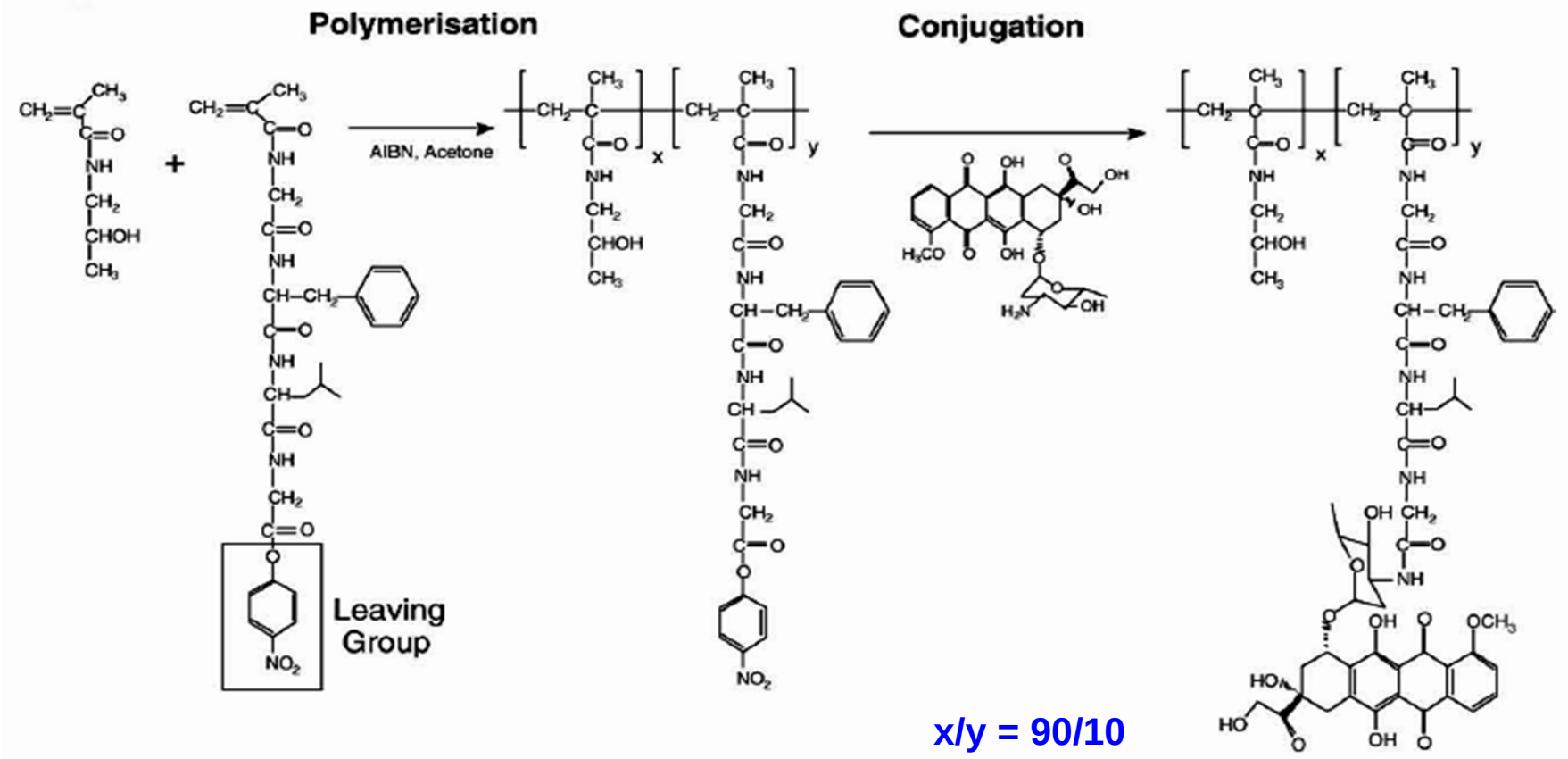
Senter et. al. *Nature Biotechnol.* **2005**, *23*, 1137-1146,
www.seattlegenetics.com

Polymer-Drug Conjugate

HPMA - N-(2-**h**ydroxy**p**ropyl)**m**ethacryl**a**mide

Developed in Czechoslovakia as a plasma expander - **Kopecek and Bazilova, 1973**

- hydrophilic
- non toxic in rats
- non-biodegradable



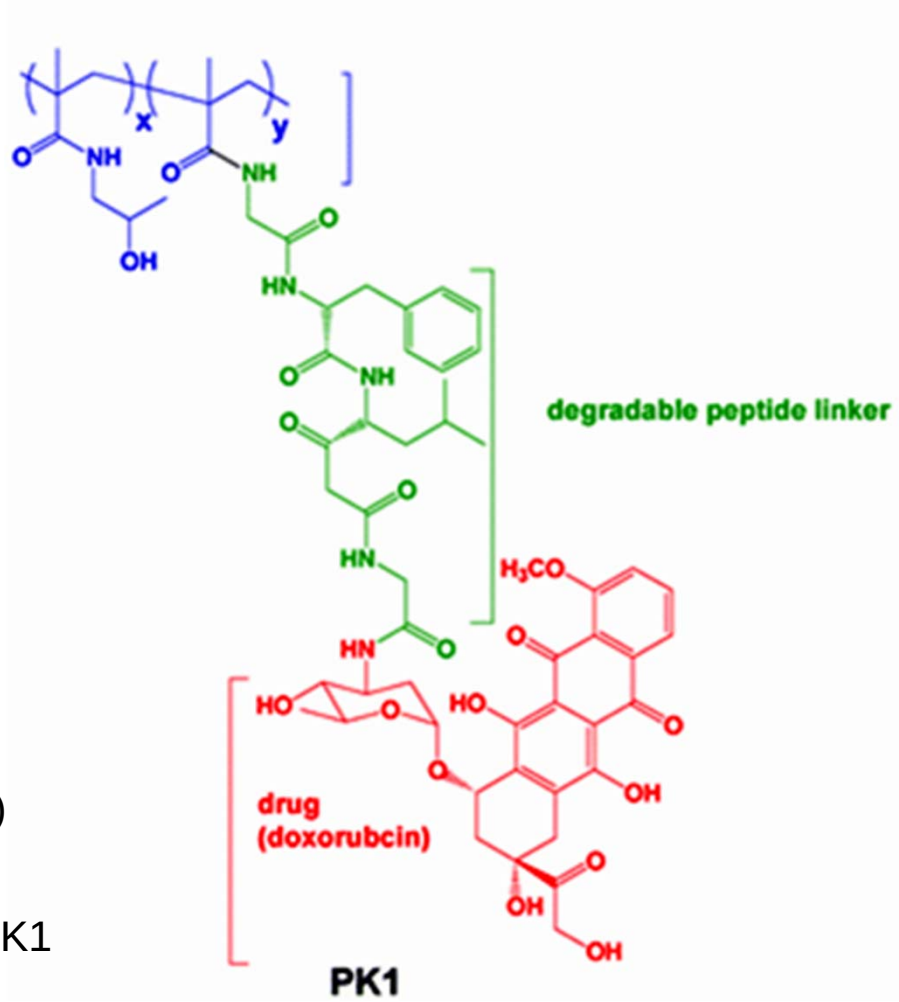
Polymer-Drug Conjugates

Diblock Co-Polymer-Doxorubicin

→ enzyme-cleavable peptide structure

PK 1 (FCE28068) - 1994

- N-(2-Hydroxypropyl)methacrylamide (HPMA) Copolymer-Doxorubicin
- Doxorrubicin content: ~ 8,5 wt %
- Tetrapeptide Linker
- EPR targeting
- Cleavage by lysosomal Cathepsin B > selective release of Doxo
- MW: 28 kDa > Excretion via kidney (barrier: 30-50 KDa)
- maximal tolerated dose: 320 mg/m^2 (10 mg/kg)
- Currently Phase II clinical trial
- **Problem**: not optimal tumor accumulation of PK1



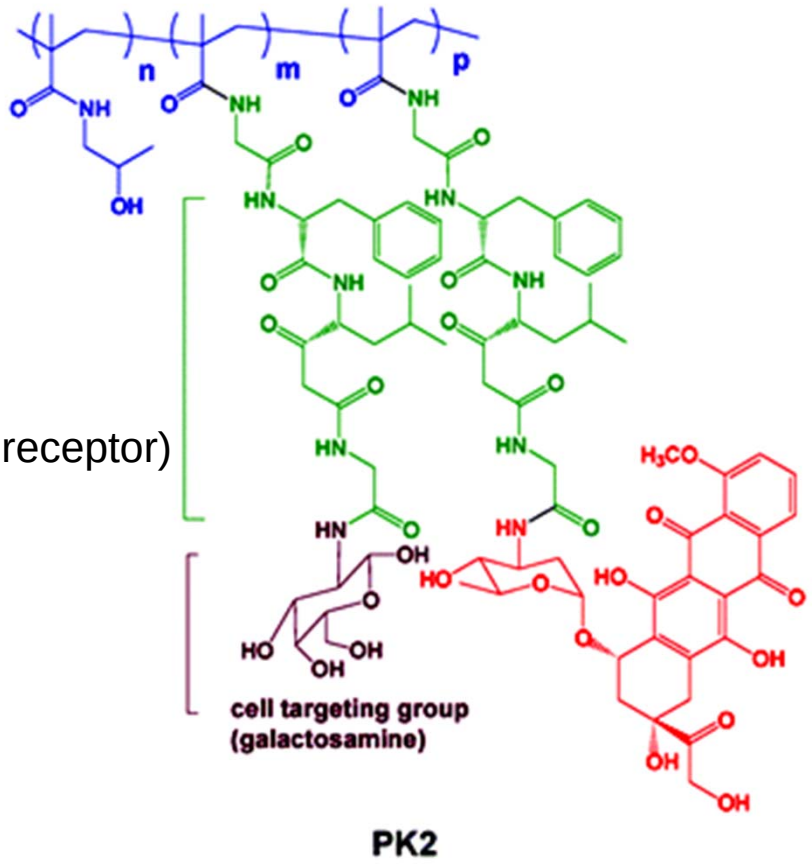
Polymer-Drug Conjugates

Diblock Co-Polymer-Doxorubicin

→ enzyme-cleavable peptide structure

PK 2 (FCE28069) - 2002

- HPMA copolymer-Doxorubicin / Galactosamine
- Doxorubicin content: ~ 8,5 wt %
- Tetrapeptide linker
- EPR Targeting
  + targeting via galactosamine (asialglycoprotein receptor)
- Cleavage by lysosomal cathepsin B
- MW: 25 kDa
- maximal tolerated Dose: 320 mg/m^2 (10 mg/kg)
- Currently Phase II clinical trial



PK2

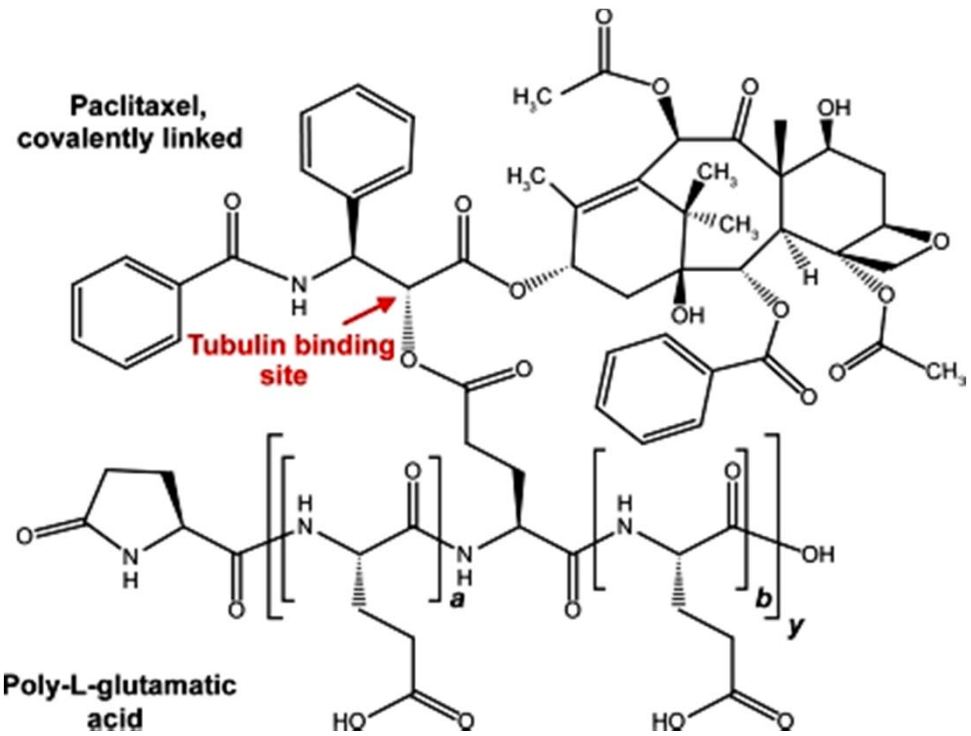
Polymer-Drug Conjugates

Diblock Co-Polymer-Taxol

→ pH-cleavable release of drug

PG-TXL (CT2103) - 1992

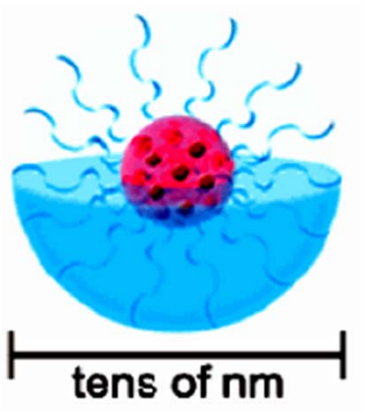
Polyglutamate-Paclitaxel

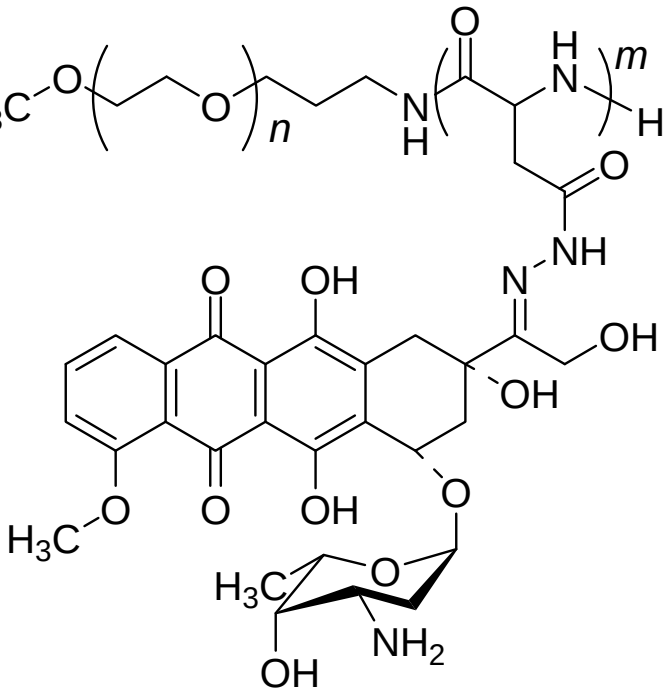


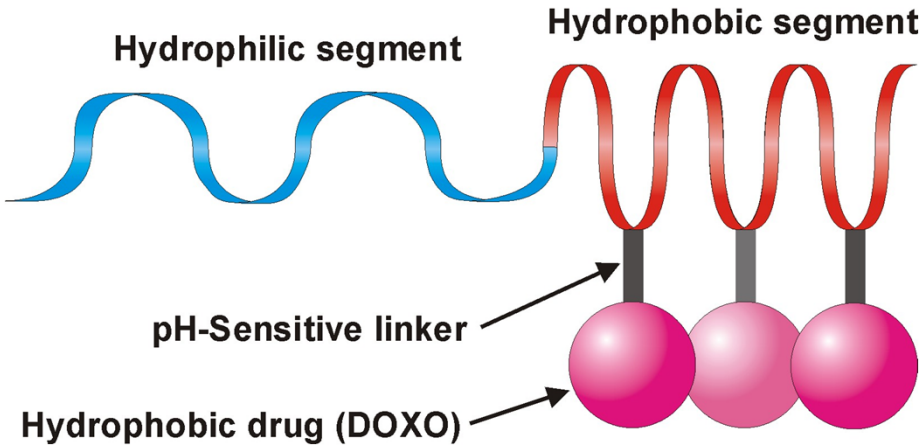
- Mixture of connection via two different OH-groups in Paclitaxel (ester bonds)
- Drug content ~ 37%
- Improved water solubility >20mg/kg
- Release by ester hydrolysis
- PG backbone is biodegradable (in vitro and in vivo -clevage by Cathepsin B)
- MTD ~ 200 mg/m^2 Taxol-eq.
- **Phase III clinical trial** (CT2103, Cell Ther.)

Polymer-Drug Conjugates

PEG-*b*-p(Asp-HYD-DOXO)

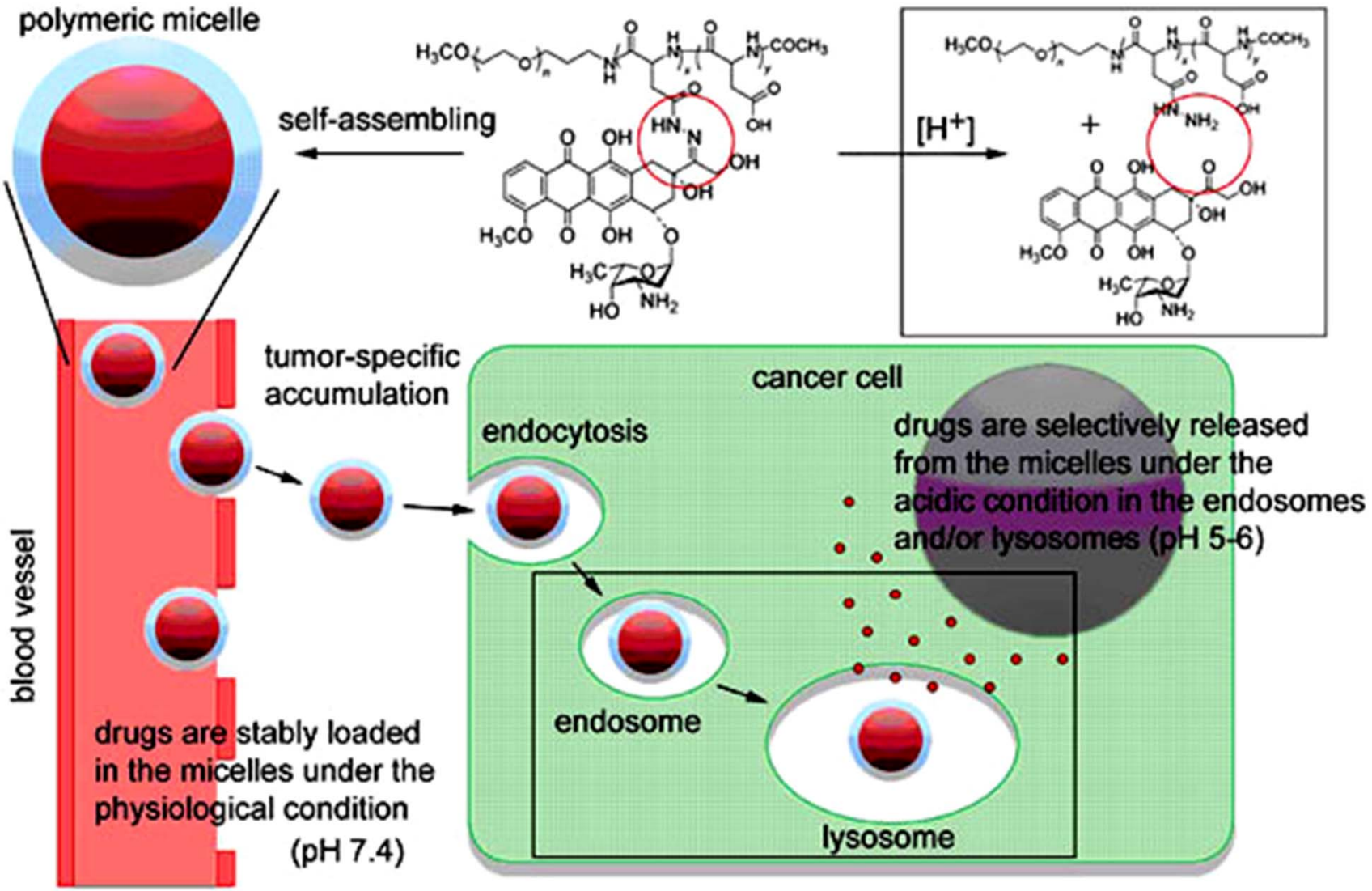






Schematic picture of block copolymer and chemical structure of PEG-*b*-p(Asp-HYD-DOXO)

PEG-*b*-p(Asp-HYD-DOXO)



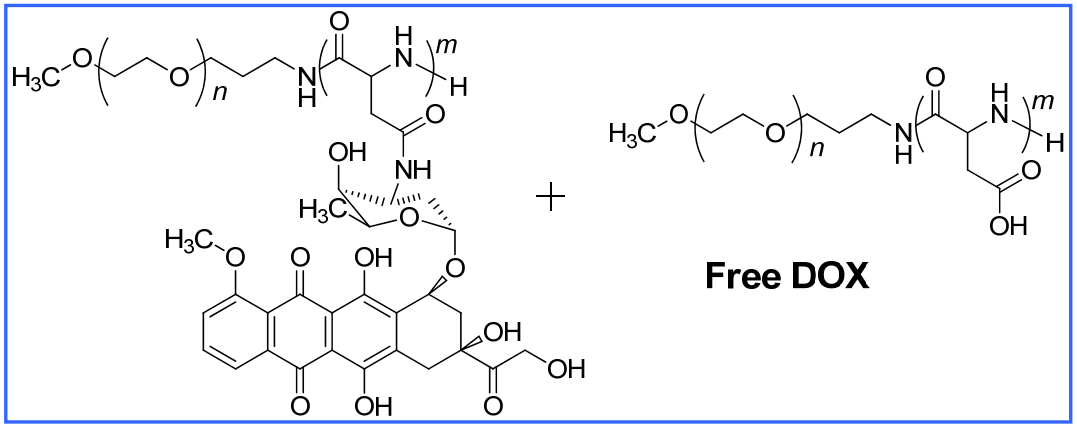
Polymeric Micelle in Cancer Therapy

NK911

• Doxorubicin-loaded PEG-b-PAsp copolymer micelle formulation (*Kataoka*)

• drug physically entrapped in the micelle core and chemically conjugated to the aspartic acid side chains of the core-forming block via amide linkages

• phase II clinical trial evaluation for efficacy and toxicity profiles



Phase I (2001):

• longer circulation half-life (50nm)

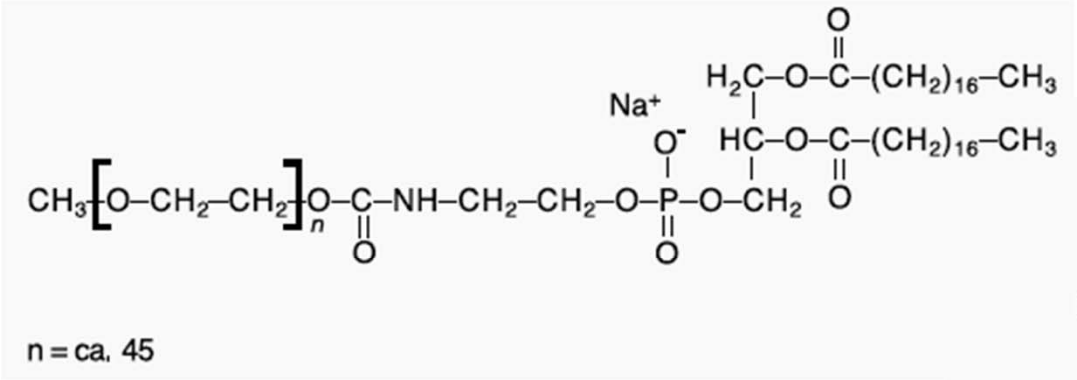
• a larger area under the curve (AUC)

• reduced toxicities in comparison to the conventional formulation of Dox

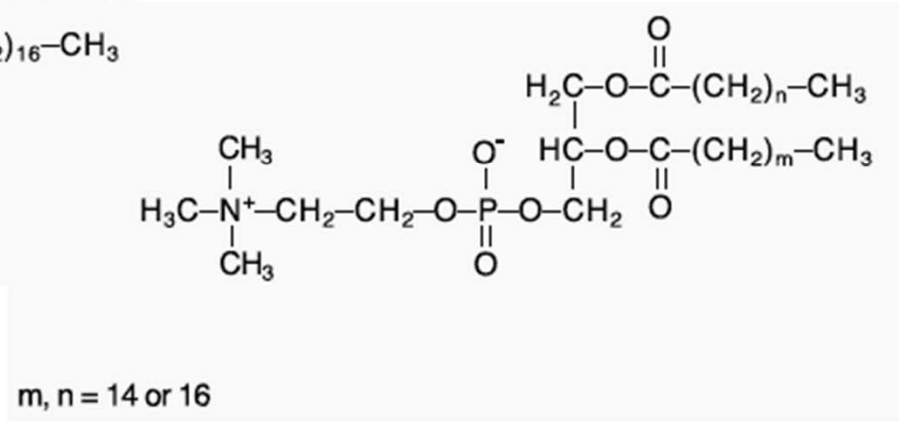
Polymeric Vesicles in Cancer Therapy

DOXIL® (doxorubicin HCl liposome injection) is doxorubicin hydrochloride (HCl) encapsulated in **STEALTH®** liposomes for intravenous administration

Compositions of the STEALTH® liposome carriers



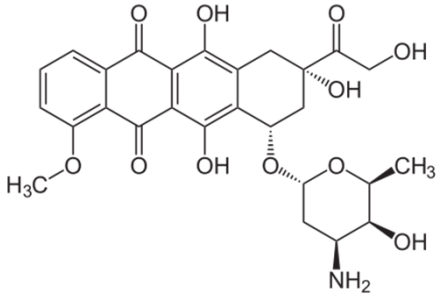
MPEG-DSPE



HSPC

Polymeric Vesicles in Cancer Therapy

DOXIL®, Caelyx® (Doxorubicin-Liposomal)



- Encapsulation of Dox through active loading that is based on an ammonium sulfate gradient
- 15000 Doxorubicin molecules per Vesikel (100 nm)
- Slow tissue clearance after injection ⇒ **PEGylation**
- Long circulation in bloodstream
- slow, targeted release of the drug
- 6-fold higher effectivity in comparison to the free Dox