Polymer Therapeutics

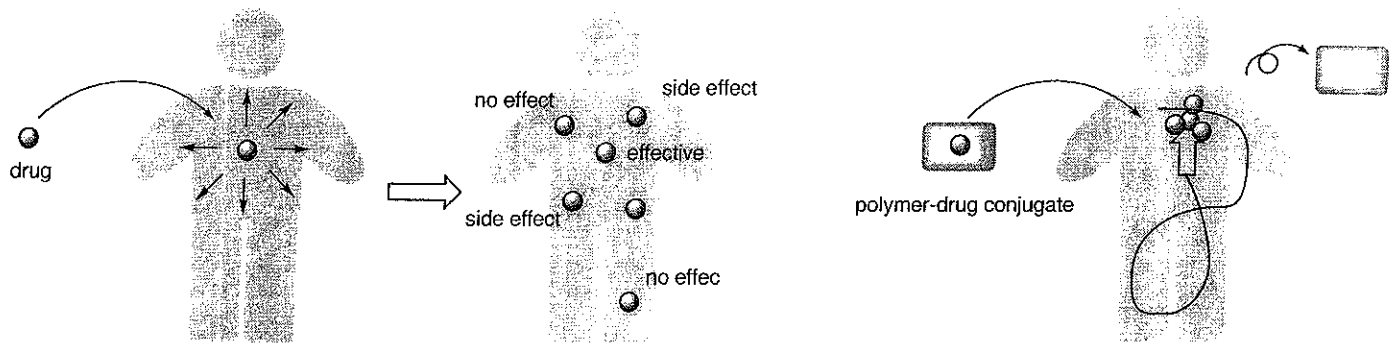
1. Introduction

Improving the therapeutic index of drugs is a major impetus for innovation in many therapeutic areas.

The vast majority of clinically used drugs are low-molecular-weight compounds that exhibit a short half-life in the blood stream and a high overall clearance rate.

They diffuse rapidly into healthy tissues and are distributed evenly within the body.

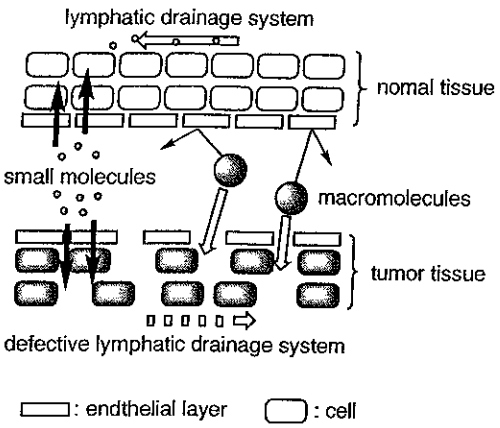
Reratively small amounts of the drug reach the target site, and therapy is associated with side effects.



A number of macromolecular delivery systems are under investigation to circumvent these limitations and improve the potential of the respective drug.

2. Biological Rationale for Macromolecular Drug -Derivery Systems

2-1. Enhanced Permeability and Retention (EPR) Effect

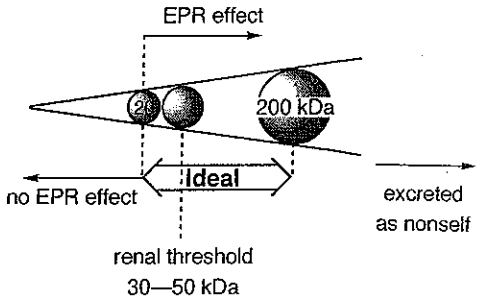


- fenestrated endothelial layer
- defective lymphatic drainage system

⇩

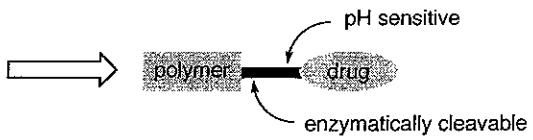
accumulation of maclomolecules

EPR effect is observed for macromolecules with molecular weights > 20 kDa and macromolecules with molecular weights in the range of 20 to 200 kDa have been selected.



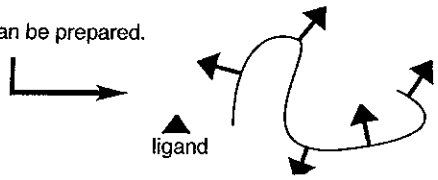
2-2. Cellular Uptake of Polymer

In general, macromolecules are taken up by the cell through endocytosis.
During endocytosis a significant drop in the pH value takes place.
A great number of lysosomal enzymes become active in the acidic environment.



2-3. Multivalent Interactions

Using polymeric spacers multivalent drugs can be prepared.



⇒ high entropic gain in the formation of the multivalent complex

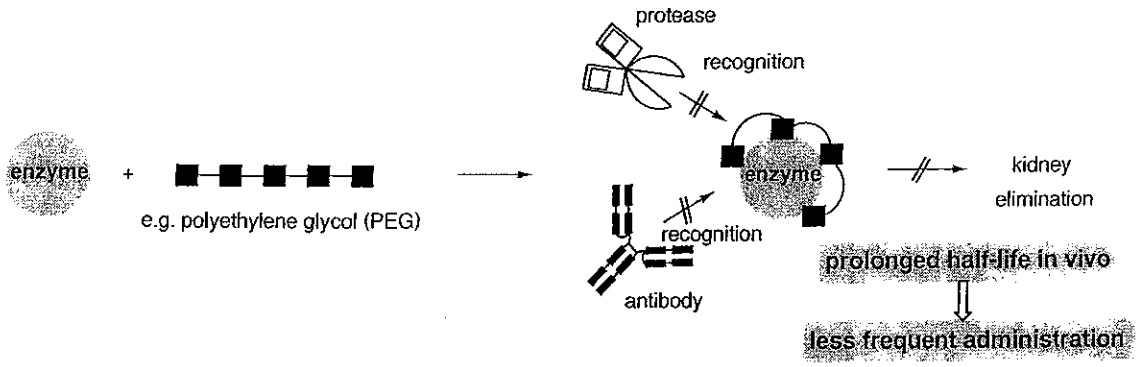
3. Approaches and Applications

3-1. Polymer Condugates of Therapeutically Relevant Proteins

PEGylation of the native protein increases its moleular weight and as a result prolongs the harf-life in vivo, which in turn allows less frequent administration of the therapeutic protein. (EPR effect and reduce the immunogenicity)

Table 1: Polymer–protein conjugates with market approval.

Trade name	Protein	Polymer	Indication	Marketed
adagen	adenosine deaminase	5 kDa PEG	severe combined immunodeficiency disease	Enzon
oncaspar	asparaginase	5 kDa PEG	acute lymphatic leukemia	Enzon
pegvisomant	GH antagonist	5 kDa PEG	excessive growth (acromegaly)	Pfizer
PEG-intron	interferon α2b	12 kDa PEG	hepatitis C	Schering-Plough
pegasys	interferon α2a	40 kDa PEG	hepatitis C	Roche
neulasta	granulocyte colony stimulating factor	20 kDa PEG	neutropenia	Amgen
SMANCS/ lipiodol	neocarzinostatin	copolymer of styrene maleic acid	hepatocellular cancer	Yamanouchi Pharmaceutical Company



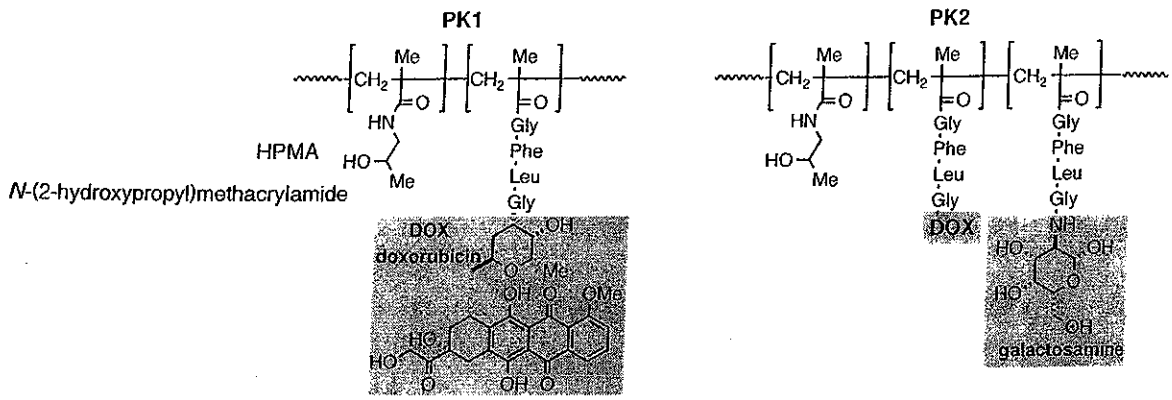
3.2 Drug-Polymer Condugates with Cleavable Linkers

The coupling of low molecular weight anticancer drugs to polymers through a cleavable linker has been an effective method for improving the therapeutic index of clinically established agents. (EPR effect and additional targeting ligand)

3-2-1. Drug-Liner Polymer Condugates

Table 2: Drug–polymer conjugates in clinical trials.

Compound	Spacer	Molecular weight [kDa]	Status of development
PK1, doxorubicin–(HPMA copolymer)	Gly-Phe-Leu-Gly	30	phase II
PK2, galactosaminated doxorubicin–(HPMA-copolymer)	Gly-Phe-Leu-Gly	30	phase I discontinued
PNU-166945, taxol–(HPMA copolymer)	ester	40	phase I completed
MAG-CPT, camptothecin–(HPMA copolymer)	Gly-6-aminohexanoyl-Gly	30	phase I completed
AP5280, diammineplatinum(II)–(HPMA copolymer)	Gly-Phe-Leu-Gly	25	phase I completed
AP5286, diaminocyclohexaneplatinum(II)–(HPMA copolymer)	Gly-Phe-Leu-Gly	25	phase I
prothecan, camptothecin–PEG conjugate	alanine ester	40	phase II
CT-2103, taxol–polyglutamate conjugate	ester	40	phase II/III
CT-2106, camptothecin–polyglutamate conjugate	Gly-ester	50	phase I
MTX-HSA, methotrexate–albumin conjugate	–	67	phase II
DOXO-EMCH, 6-maleinimodcaproyl hydrazone derivative of doxorubicin	acid-sensitive hydrazone	67 (albumin-bound prodrug)	phase I completed



3-2-2. Drug-Dendrimer Condugates

cf. What is dendrimer

dendri (樹木状) + meros (一部) = Dendrimer

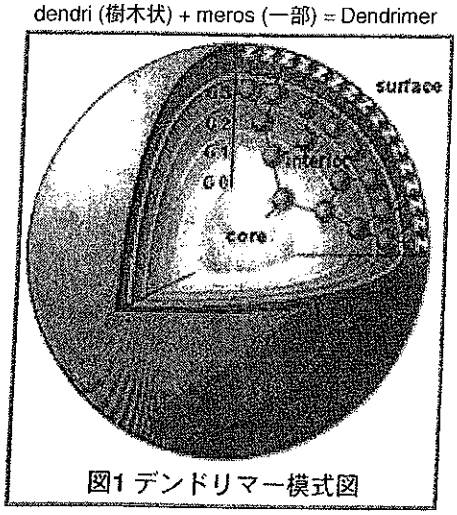


図1 デンドリマー模式図

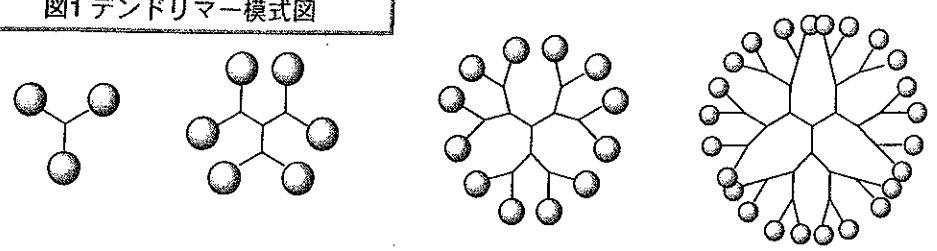
Dendrimer consists of core, interior and surface.

core: total size, shape, directionality

interior: inner character, size

surface: various reactive or unreactive unit

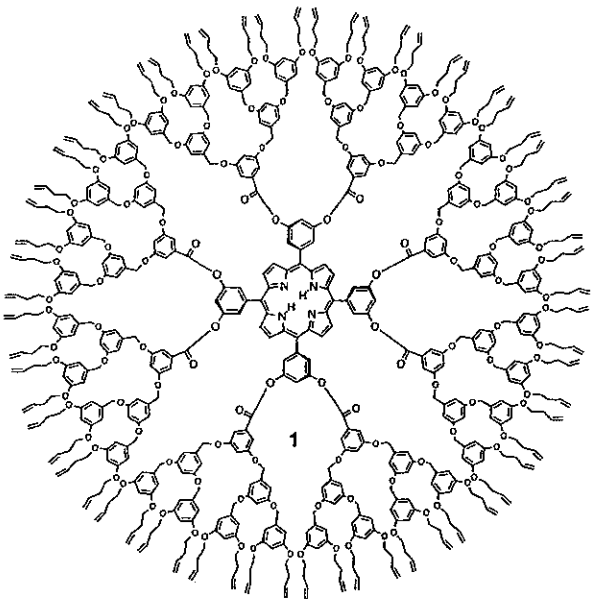
The structural control of dendrimers is easier than general macromolecules.

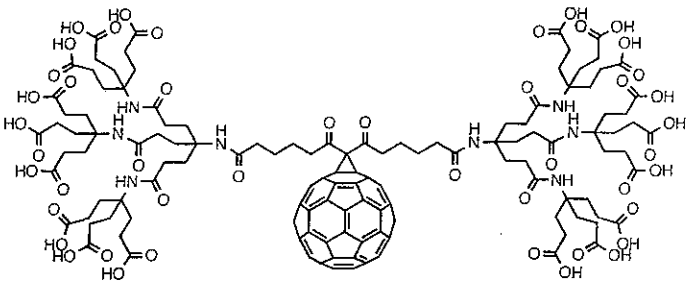


increase linearly for generation

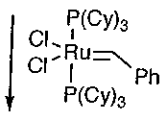
G (generation) = 0 G = 1 G = 2 G = 3

= 3 = 6 = 12 = 24 ← increase exponentialy for generation



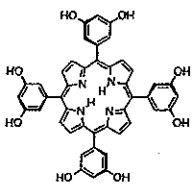


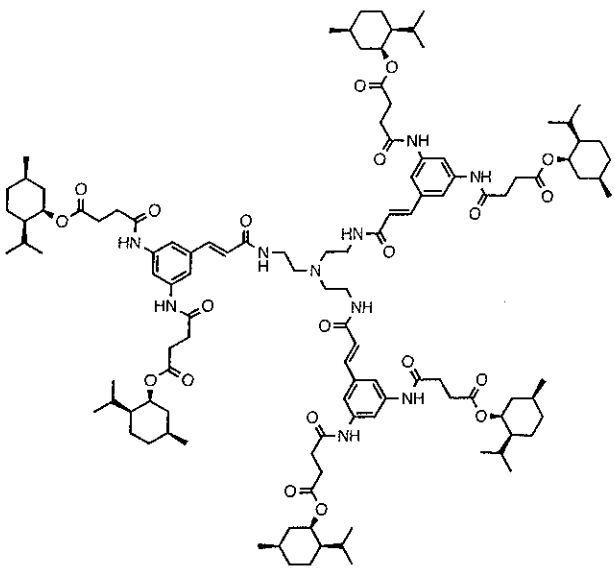
Fullerodendrimer: Hirsch et al. (See Hara's literature seminar)



Molecular imprinting: Zimmerman et al.

, benzene





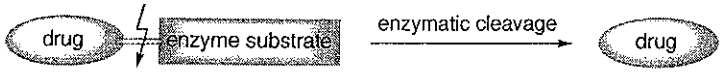
Fragrance-release dendrimer: Youngs et al.

Single-Triggered Trimeric Prodrugs

Doron Shabat et al. *Angew. Chem. Int. Ed.* **2005**, *44*, 716.

Concept and General Structure of a Sngle-Triggered Trimeric Prodrug

drug:enzyme substrate = 1:1 condugate

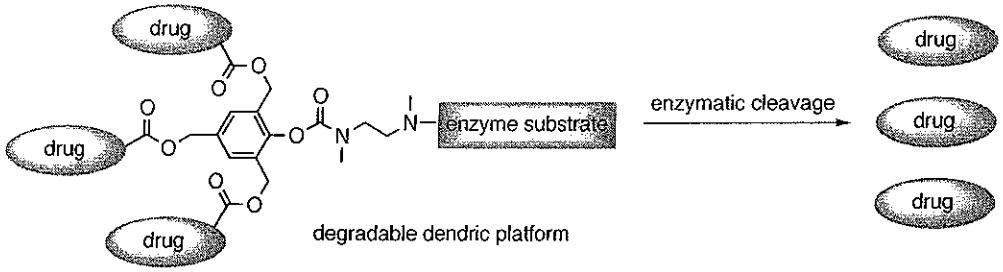


one enzymatic reaction can release one drud.

⇩

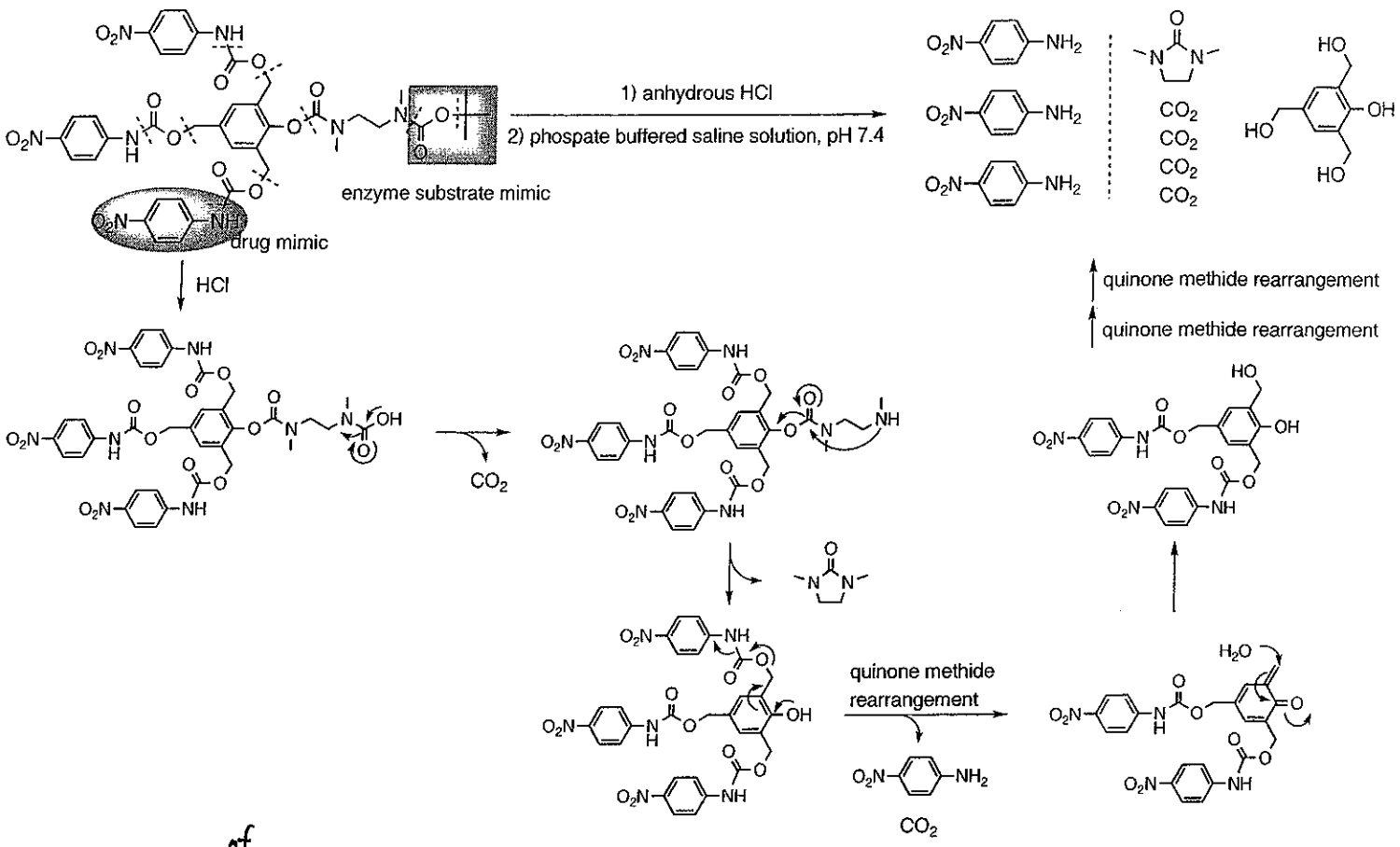
The amount of drug activated at the targeted tissue is dependent on the rate and concentration of the specific enzyme.

drug:enzyme substrate = 3:1 condugate

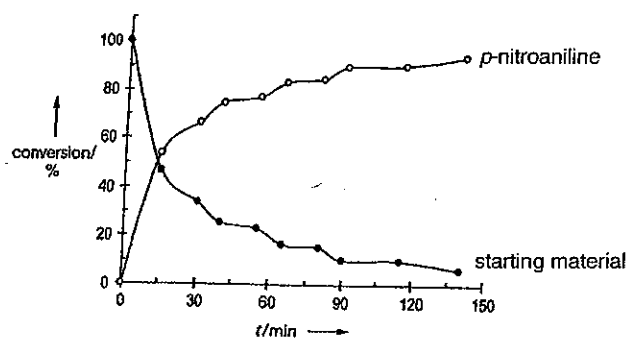


Single enzymatic cleavage can release (more than) three drug molecules.

Model System



Release (% conversion) ~~of~~ p-nitroaniline from the trimeric platform

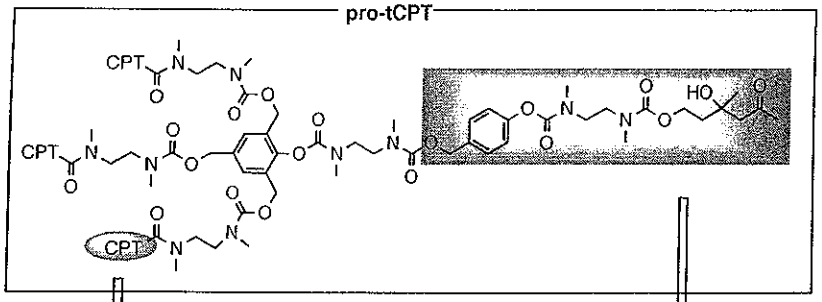


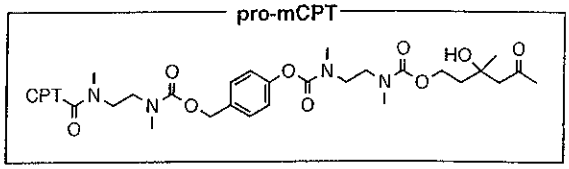
← The progress of the reaction was monitored by HPLC by following the formation of p-nitro-aniline.

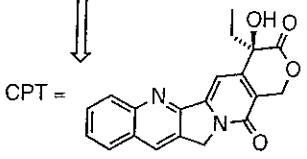
(No release was observed when the Boc group remained attached to the platform.)

→ Self-cyclization and triple quinone methide rearrangement could indeed take place under physiological conditions.

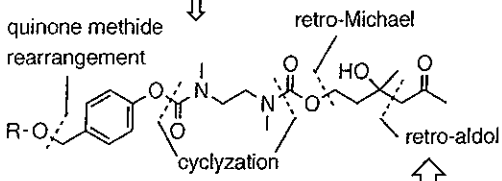
Trimeric Prodrug System







camptothecin



catalytic antibody 38C2: catalyzes aldol and retro-aldol reactions.

The ability of the prodrugs to inhibit cell proliferation in the presence of catalytic antibody 38C2.

IC_{50} values [nM] from cell-grouth inhibition assay.

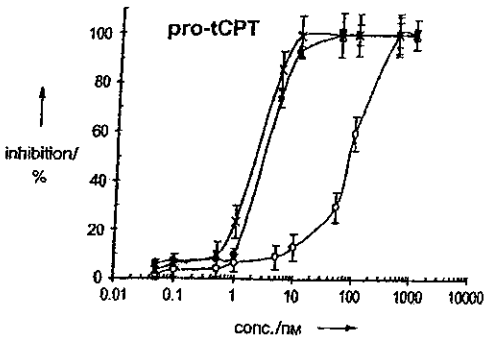
Drug/Prodrug	MOLT-3		HL-60		HEL	
	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}	IC_{50}
CPT	2.2	2.0	9.0	7.5	13	11
pro-mCPT	100	15	150	31	400	100
pro-tCTP	80	2.7	100	7.5	200	19

without catalytic antibody 38C2

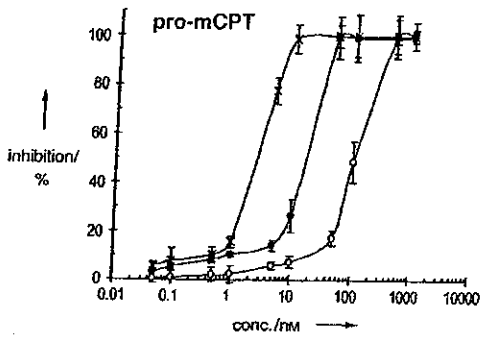
with 1-μM catalytic antibody 38C2

MOLT-3: humane T-lineage acute lymphoblastic leukemia cell line
HL-60: humane acute myeloid leukemia cell line
HEL: humane erythroleukemia cell line

Data from the MOL-3 cell line

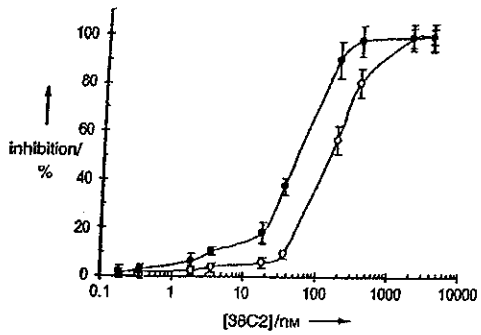


○ = pro-tCPT
● = pro-tCPT + 38C2
× = CPT



○ = pro-mCPT
● = pro-mCPT + 38C2
× = CPT

Growth inhibition assay of the human MOLT-3 leukemia cell line at a fixed concentration of the prodrug and upon variation of the concentration of the catalytic antibody 38C2

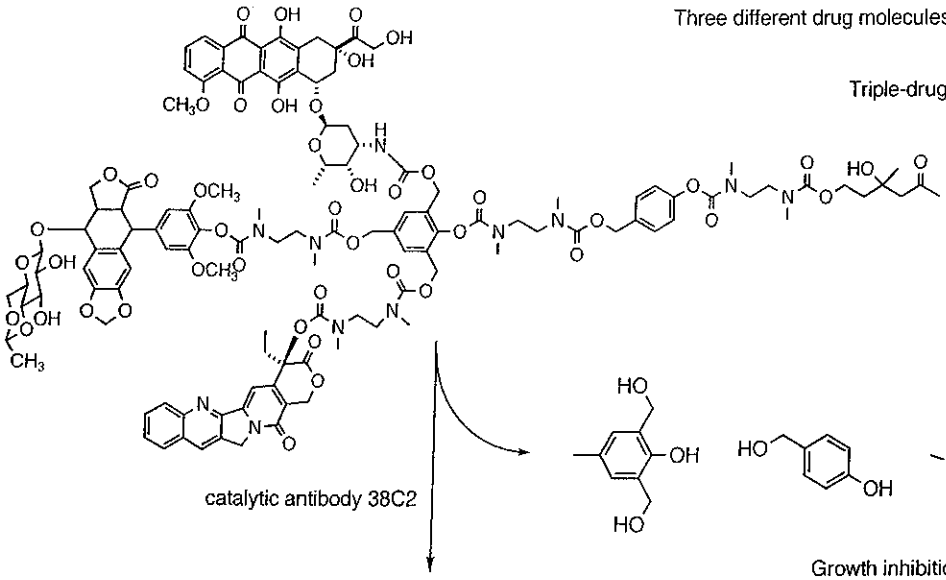


○ = 36-nM pro-mCPT
● = 12-nM pro-tCPT

To have equal amount of CPT, the concentration of the monomeric prodrug was three times that of the trimeric prodrug.

The concentration of antibody needed to inhibit the cell growth by 50% with pro-tCPT is about three times less than that needed with pro-mCPT.

Single-Triggered Heterotrimeric Prodrug System

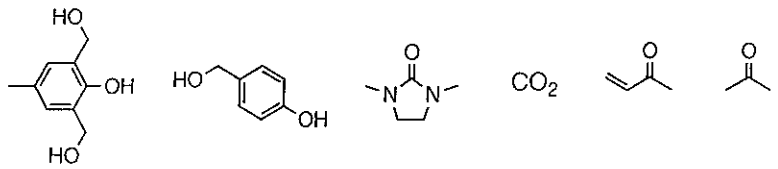


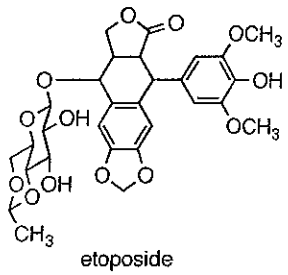
Three different drug molecules are incorporated on the same prodrug platform.

⇩

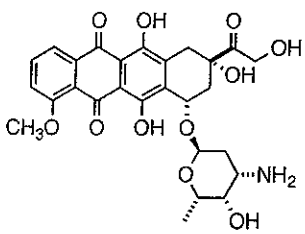
Triple-drug therapy in a single molecule

catalytic antibody 38C2

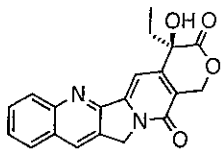




etoposide

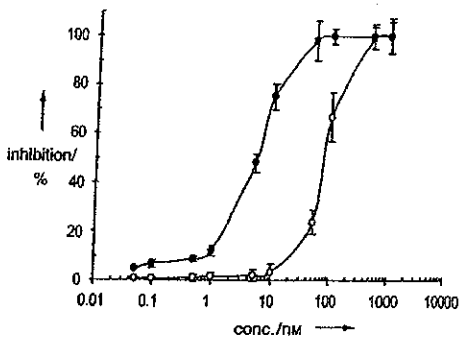


doxorubicin



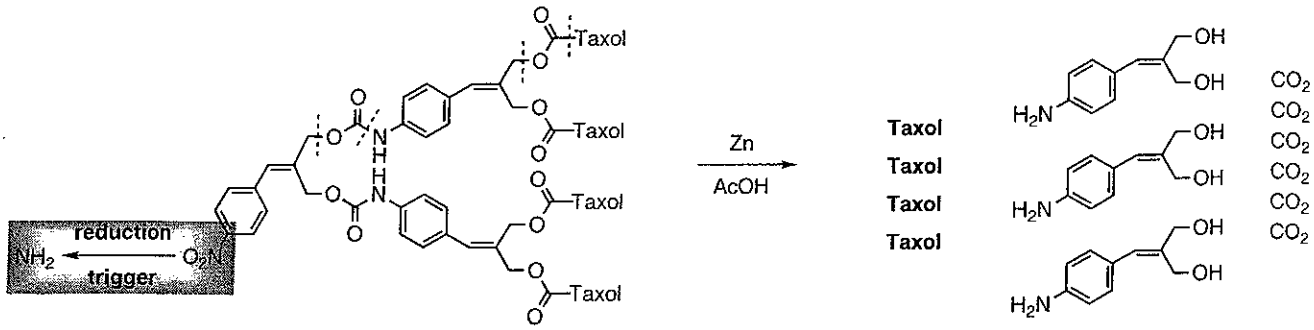
camptothecin

Growth inhibition assay of the human MOLT-3 leukemia cell line.



The inhibition by the heterotrimeric prodrug was increased approximately 15-fold on activation by antibody 38C2

Franciscus M. H. de Groot et al. *Angew. Chem. In. Ed.* **2003**, *42*, 4490.

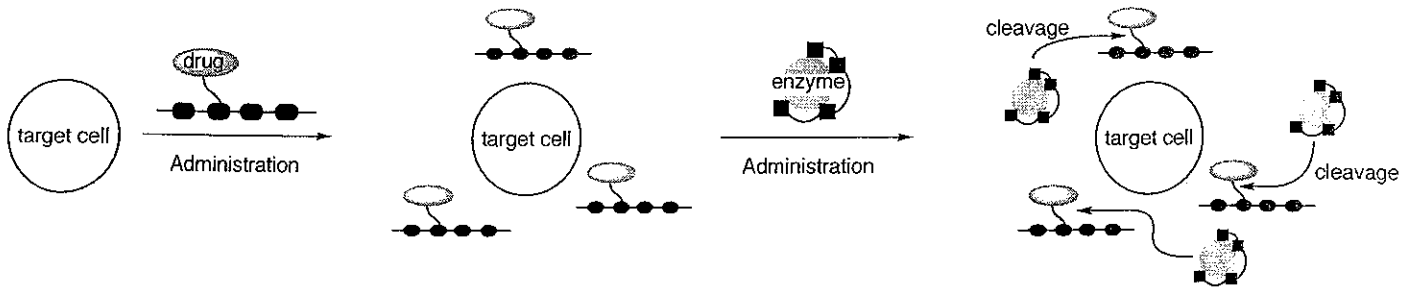


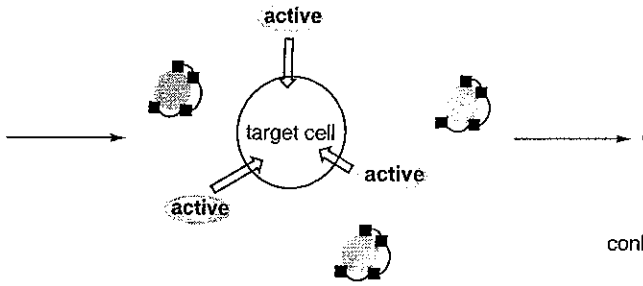
3-3. A Combined Approach: The PDEPT Concept

PDEPT (polymer-directed enzyme-prodrug therapy) is two-step antitumor approach that combines a polymeric prodrug and polymer-enzyme conjugate to generate a cytotoxic drug at the tumor site.

polymer-enzyme conjugate

polymer-drug condugate (prodrug)

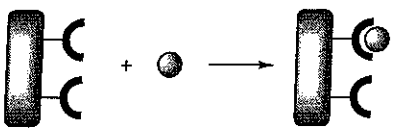




The antitumor activity (the survival time of treated/control animal)

conbination of PK1 (see p. 2) and HPMA-cathepsin B (protease) : PK1 alone = 1.68

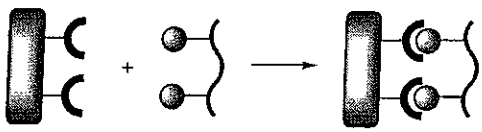
3-4. Maltivalent Therapeutics



binding constants

bivalent interactions: > 1000 than monovalent binding

tri~pentavalent interactions: ~10^8 have been reported

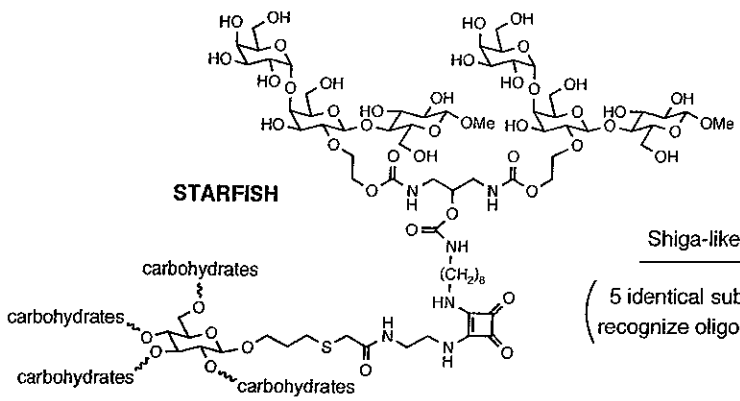


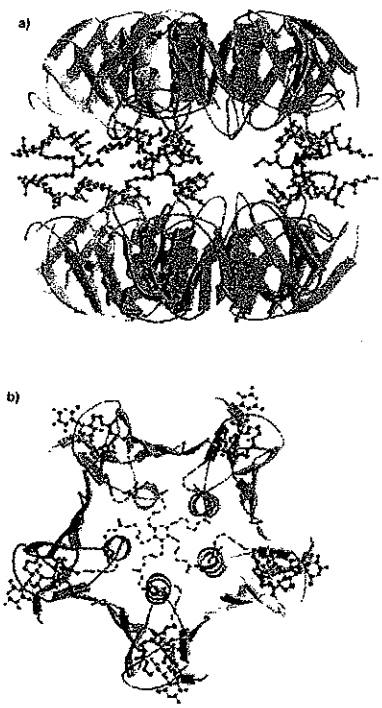
target

dimeric or multimeric proteins

cell surface receptor, inhibition of virus

DNA/RNA transfection agents





An increase in the binding affinity by a factor of 10^7 was observed relative to monovalent ligand.

Figure 10. Pentavalent binding of the multivalent polysaccharide inhibitor to the Shiga-like toxin dimer: a) side view, b) top view (adapted from ref. [88]).

3-5. Supromolecular Drug-Polymer Complexes

3-5-1. Block Copolymer Mielles

Block copolymer micelles are generally more stable than micells of small surfactant molecules.

⟹ retain the loaded drug for a longer period of time.

-A-A-A-A-B-B-B-B-
block copolymer

-A-B-A-B-A-B-A-B-
alternating copolymer

-A-B-B-A-B-A-A-A-
random copolymer

- hydrophilic - hydrophobic - (drug, drug) —in H_2O→

■ = active agent (e.g. drug, fluorescent dye)

= targeting group (e.g. oligosaccharide)

ca. 10–50 nm

block copolymer micelles

Kataoka et al. *Angew. Chem. Int. Ed.* **2003**, *42*, 4640.

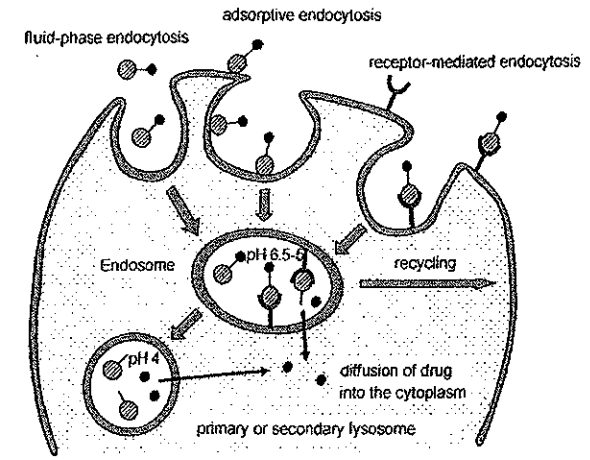
The block copolymer micelles are 65 nm in diameter

PEG-p(Asp-Hyd-Dox)

H^+

Doxorubicin

PEG-p(Asp-Hyd)



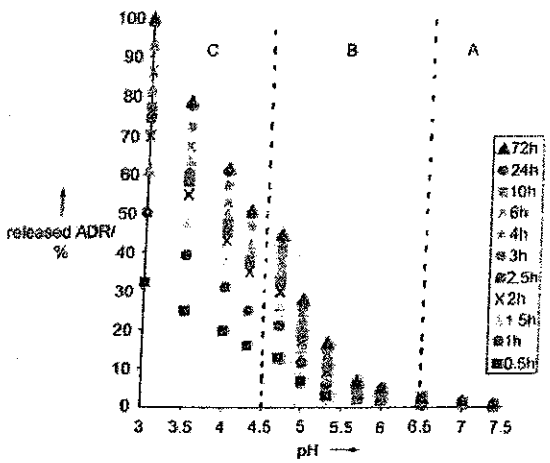
pH 7.2—7.4

extracelluar space: pH 6.5—5.0 ⟹ Design of pH-responsive drug derivary system.

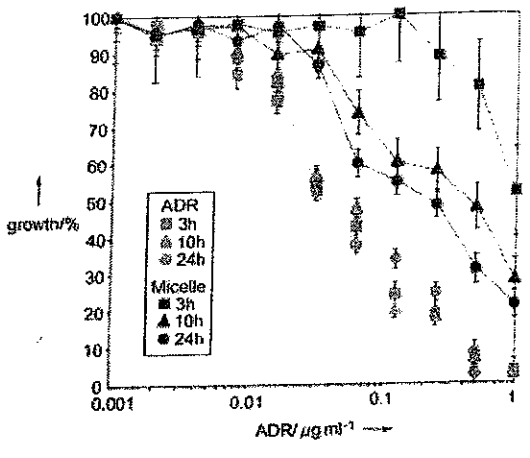
lysosome : pH ~4.0

Figure 4. Endocytotic pathway for the cellular uptake of macromolecules and nanocarriers for drug delivery.

Time and pH-dependent doxorbicin release profile



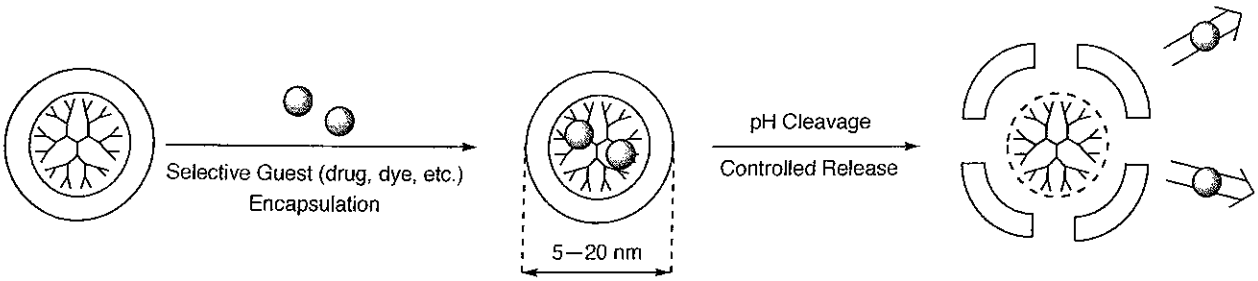
Growth inhibition assay results on human small lung cancer cells SBC-3



pH-Responsive Molecular Nanocarriers Based on Dendritic Core-Shell Architectures

Rainer Haag et al. *Angew Chem Int Ed.* **2002**, *41*, 4252.

Small surfactant molecule can be unstable under shear force and other kinds of environmental effects as a result of their weak assembly. The covalent modification of dendritic macromolecules with an appropriate shell results in stable micelle-type structures.



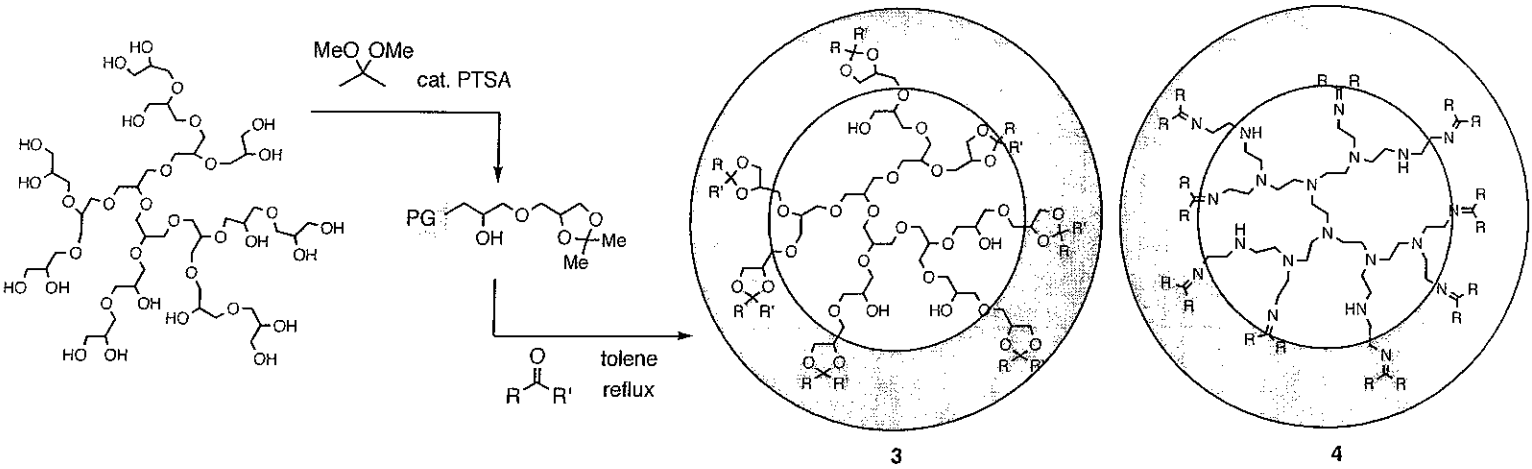


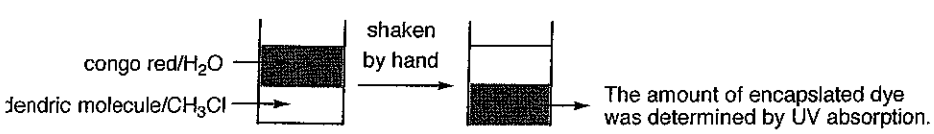
Table 1. Sizes and transport capacities of dendritic nanocarriers 3 and 4.

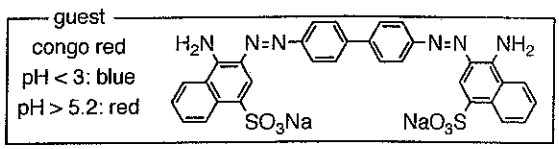
Structure	Polymer core	M_n core [g mol^{-1}]	Shell	Degree of alkylation	Size[a] [nm]	Height[a] [nm]	Transport capacity[b]
1	PG	21000	–	–	8 ± 2	2.7 ± 0.6	–[c]
3a	PG	21000	$H_{31}C_{15}$–C(=O)–	25%	10 ± 2	2.5 ± 0.5	0.15 ± 0.05
3b	PG	21000	$H_{33}C_{16}$–C(=O)–$C_{16}H_{33}$	45%	10 ± 2	2.7 ± 0.6	13 ± 4
3c	PG	21000	$H_{33}C_{16}$–C(=O)–$C_{16}H_{33}$	55%	10 ± 2	2.5 ± 0.5	2 ± 0.5
2	PEI	25000	–	–	18 ± 4	3.2 ± 0.5	0.02 ± 0.005[d]
4a	PEI	25000	$H_{31}C_{15}$–C(=O)–	33%	20 ± 4	3.5 ± 0.5	0.6 ± 0.1[d]
4b	PEI	25000	$H_{11}C_5$–C(=O)–C_5H_{11}	53%	84 ± 16	9 ± 2[e]	0.2 ± 0.05[d,e]

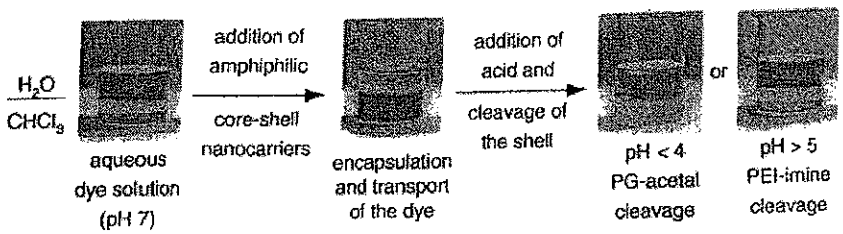
[a] Corrected particle diameter and height without encapsulated guest molecules from AFM data (see Supporting Information). [b] Number of encapsulated dye molecules (congo red) per polymeric nanotransporter and transport into the chloroform phase. [c] Not soluble in chloroform. [d] Loading capacities were determined in chloroform without aqueous phase by using a UV calibration curve. [e] Partial hydrolysis and aggregate formation is possible with water.

For efficient transport the degree of alkylation should be about 45—50%.

The transport capacities (amount of guest molecules/dendric molecule) were determined using congo red as a model compound.



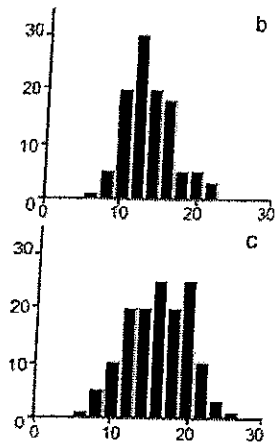




Encapsulation and transportation of polar guest molecules with dendric nanocarriers into the organic phase.

Cleavage of the shell leads to the release of the encapsulated guest back to the aqueous phase.

The particle sizes of these nanocarriers were determined by atomic force microscopy (原子間力顕微鏡, AFM)



unloaded state: 10±2 nm

swelling the polumer core upon encapsulation of the guest molecules.

dye-loaded state: 13±2 nm

Jean M. J. Frechet et al. *Bioconjugate Chem.* **2005**, *16*, 361.