NanoBio Integration for Medical Innovation

-Targeting Therapy by Supramolecular Nanodevices-

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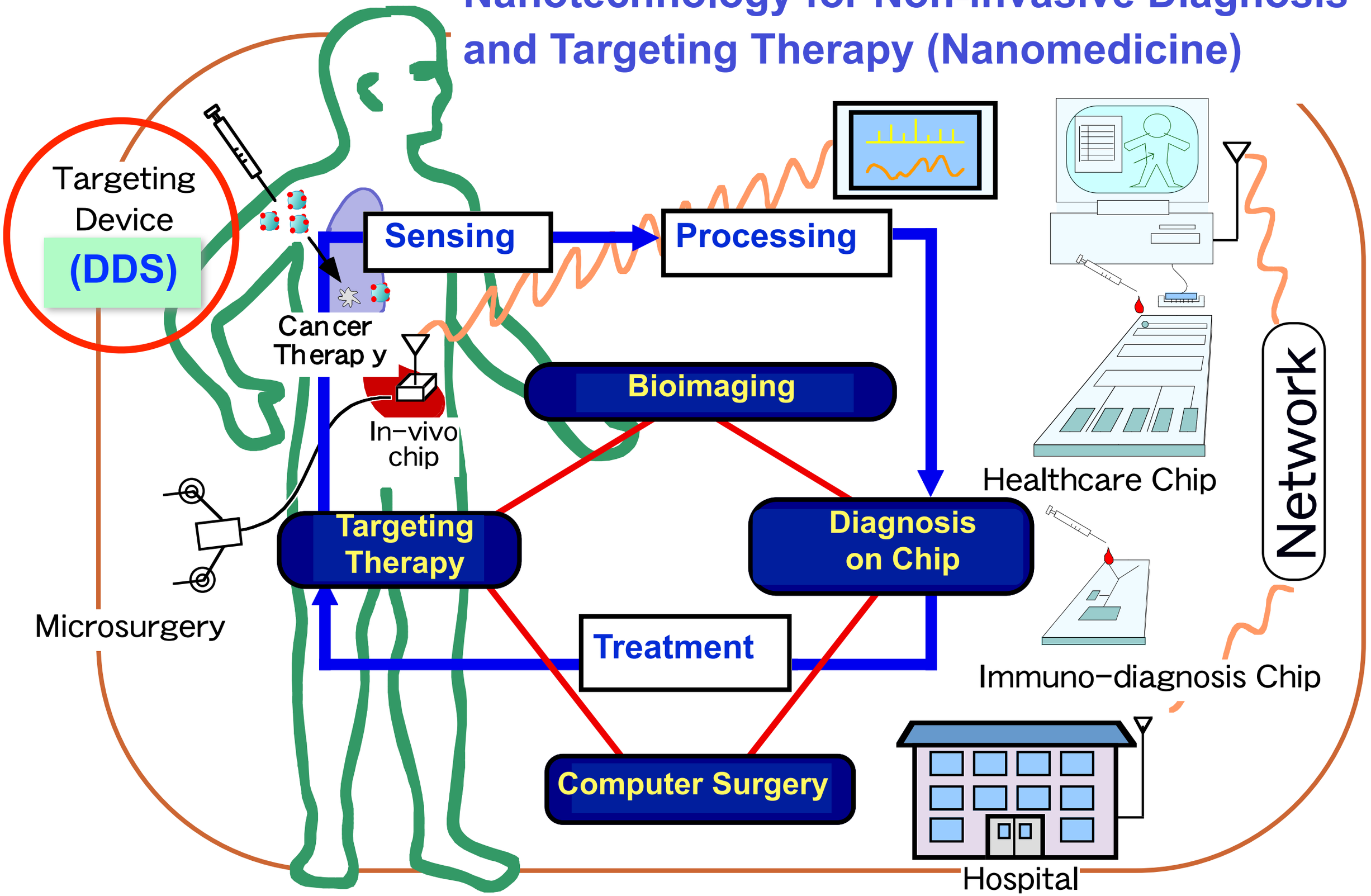
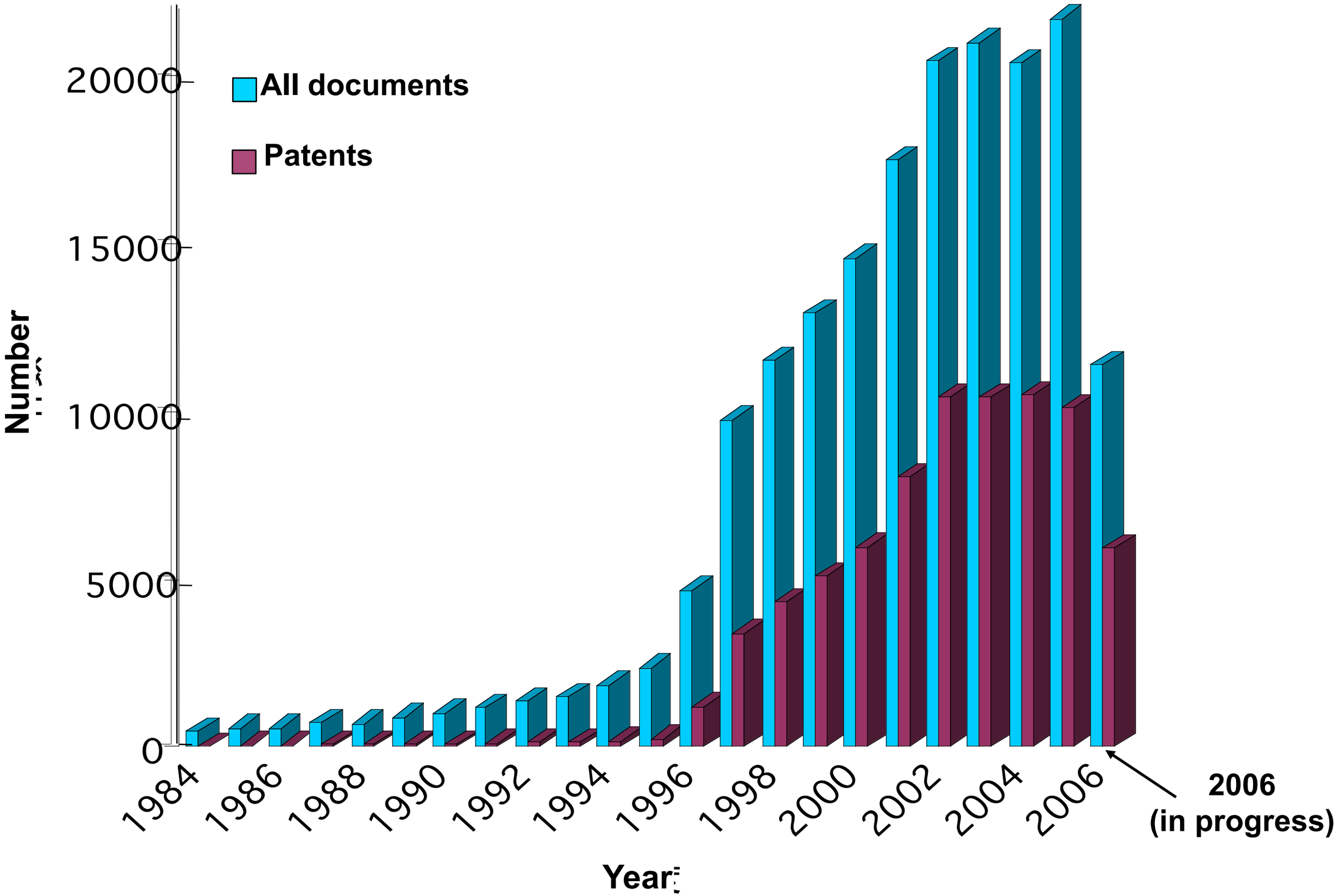
November 25, 2009
UT-SNU Exchange Lecture Course
Introduction to Bioengineering

CNBI
Center for NanoBio Integration

Nanotechnology for Non-invasive Diagnosis and Targeting Therapy (Nanomedicine)

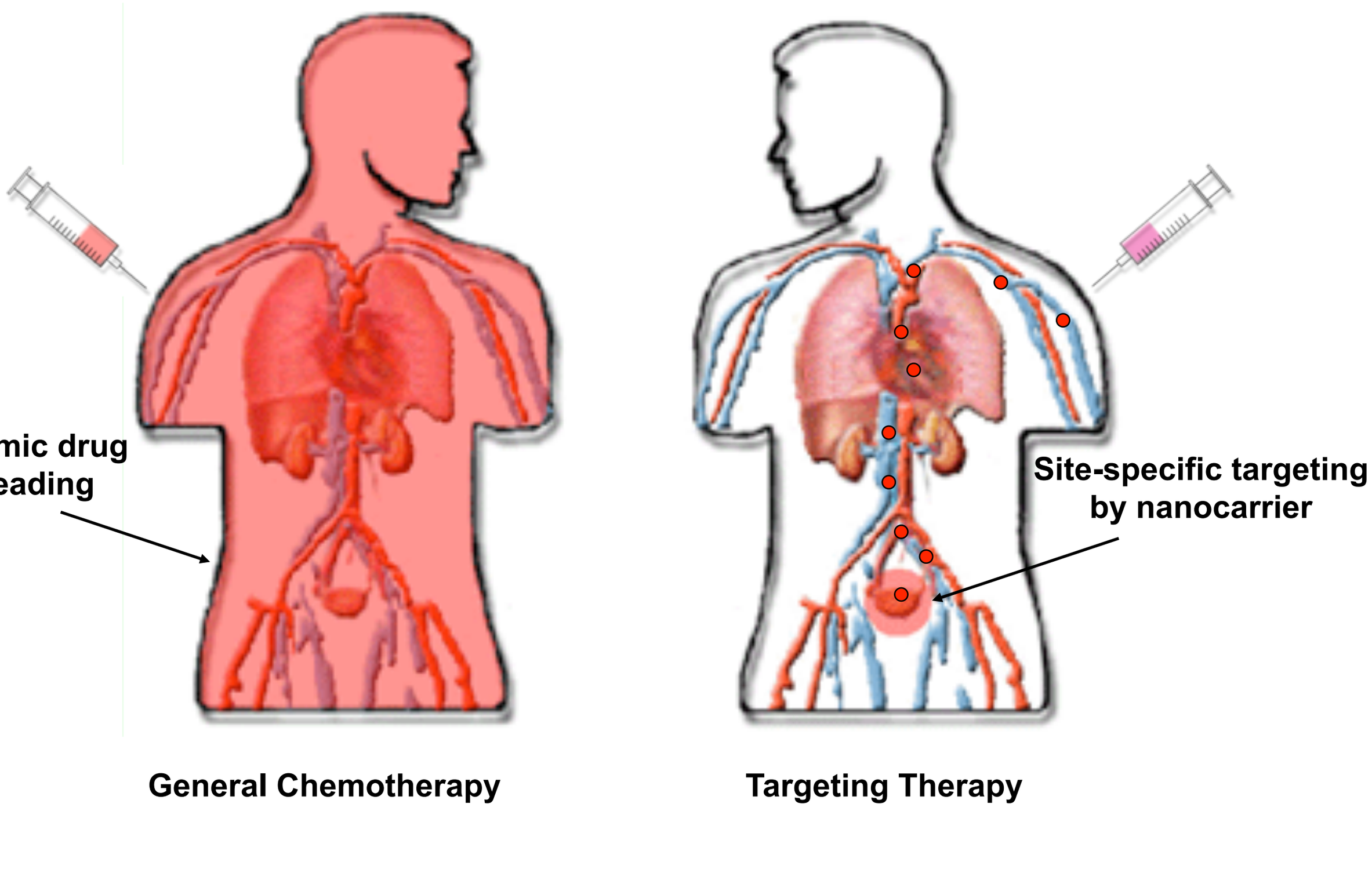


Number of scientific papers and patents related to DDS



*Source: SciFinder

Targeting Therapy by Nanotechnology-based Medicine



General Chemotherapy

Targeting Therapy

Itinerary of intravenously-injected nanocarriers

Intravenous injection of nanocarrier

i.v. injection

liver uptake

blood stream

RES

kidney

Longevity in blood circulation (Stealth property)

Effective extravasation at target site (size may be less than 100nm)

Selective uptake into target cell

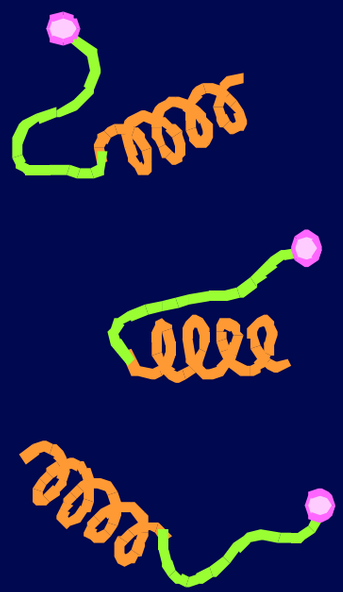
Effective trafficking into cytoplasm

Achievement of targeted therapy

Structural Design of Polymeric Micelles for DDS

Hydrophilic outer shell composed of flexible tethered polymer strands
➡Biocompatibility

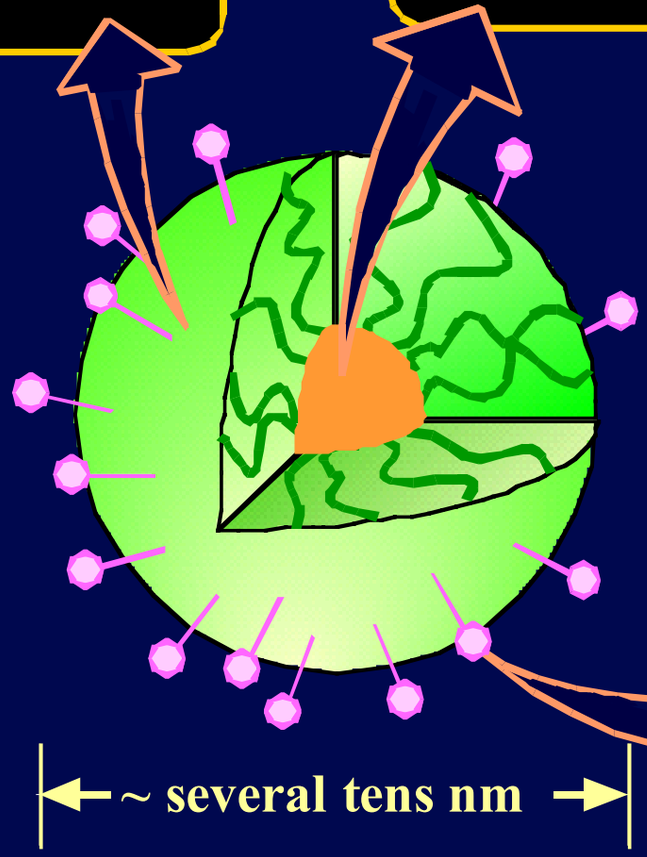
Core segregated from outer environment
➡Drug reservoir



Amphiphilic block copolymer

Multi-molecular association in aqueous milieu (association number: ~10^2)

Formation of polymeric micelle with core-shell architecture



Introduction of multi-ligands on micelle periphery
➡Targetability

Uniform size with a range comparable to viruses
➡High extravasating ability

M. Yokoyama, K. Kataoka, et al, *J. Contrl. Rel.* 11, 269 (1990); K. Kataoka, G. S. Kwon et al, *J. Contrl. Rel.* 24, 119 (1993); G. S. Kwon, K. Kataoka, et al, *J. Contrl. Rel.* 29, 17 (1994); A. Harada, K. Kataoka, *Science* 283, 65 (1999)

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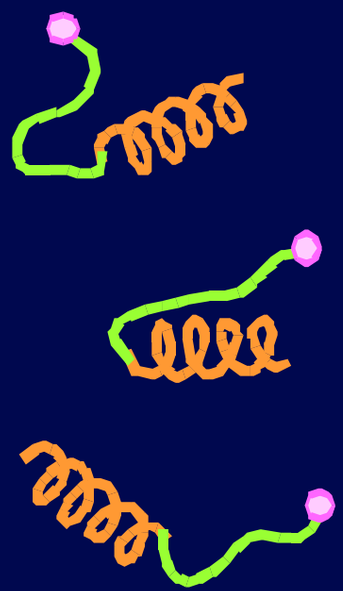

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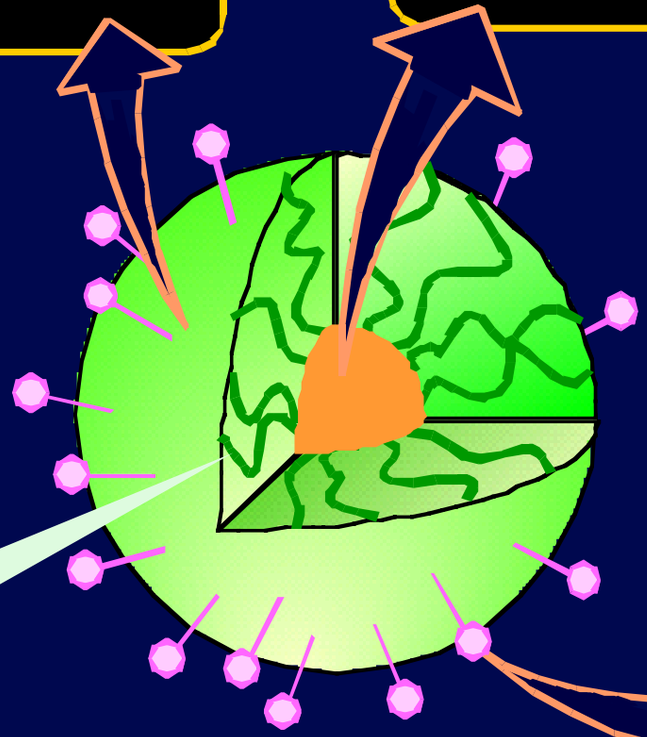

~ several tens nm

-$(CH_2CH_2O)_n$-
Poly(ethylene glycol)

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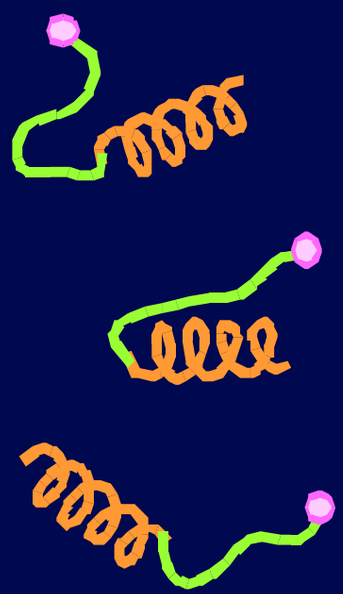

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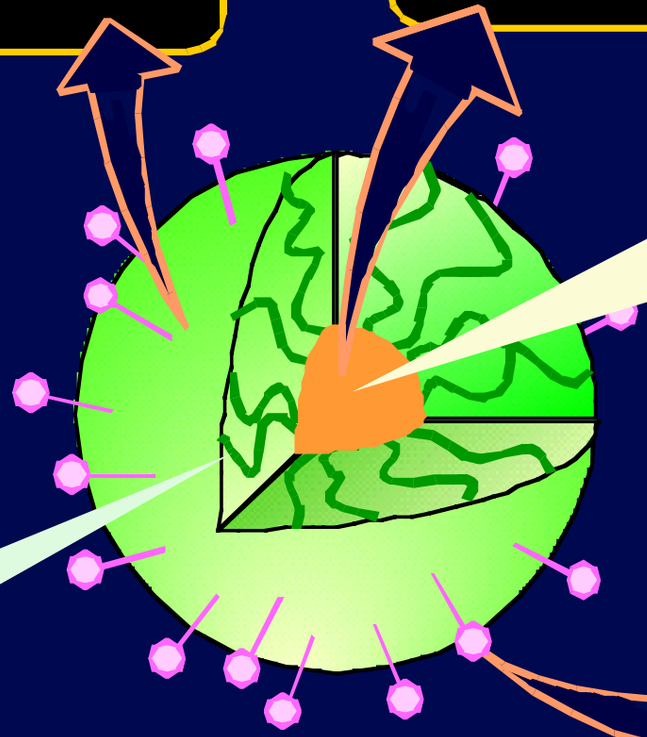
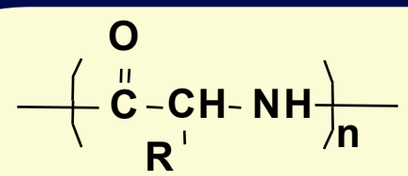


~ several tens nm



Poly(amino acid)s

-$(CH_2CH_2O)_n$-
Poly(ethylene glycol)

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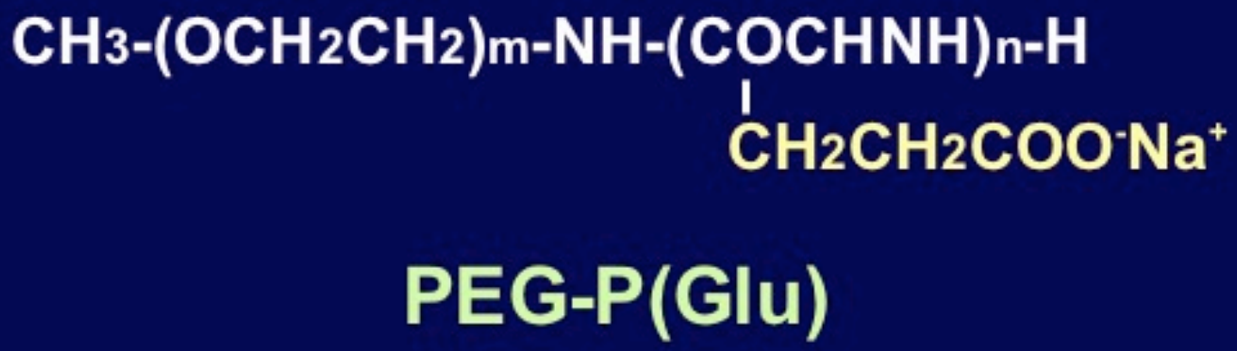
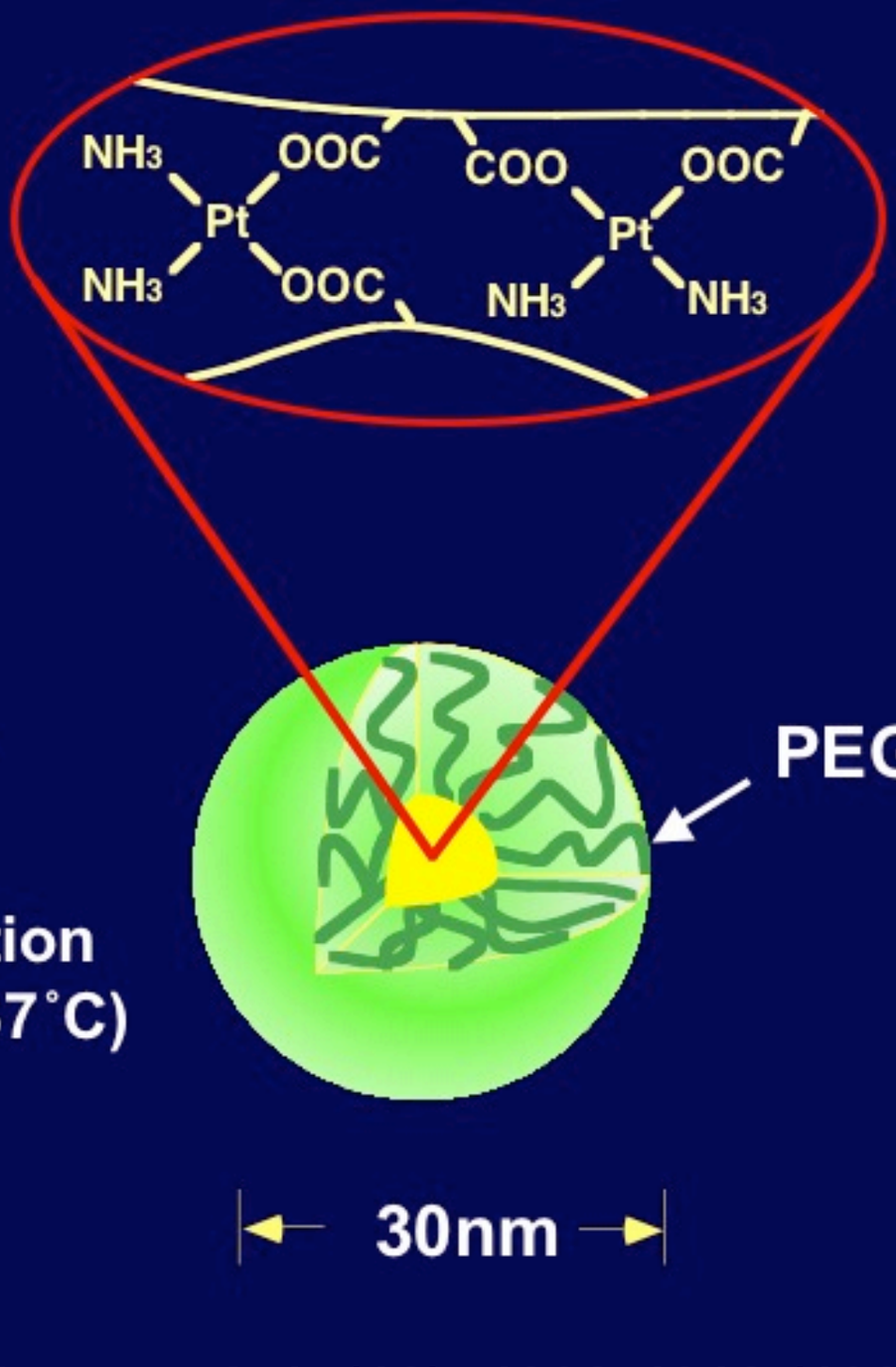
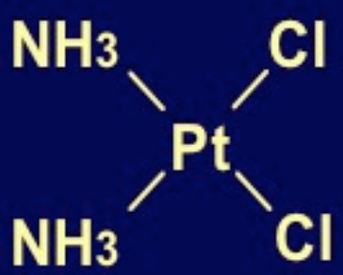
Preparation of cisplatin (CDDP)-incorporated polymeric micelles



PEG-P(Glu)





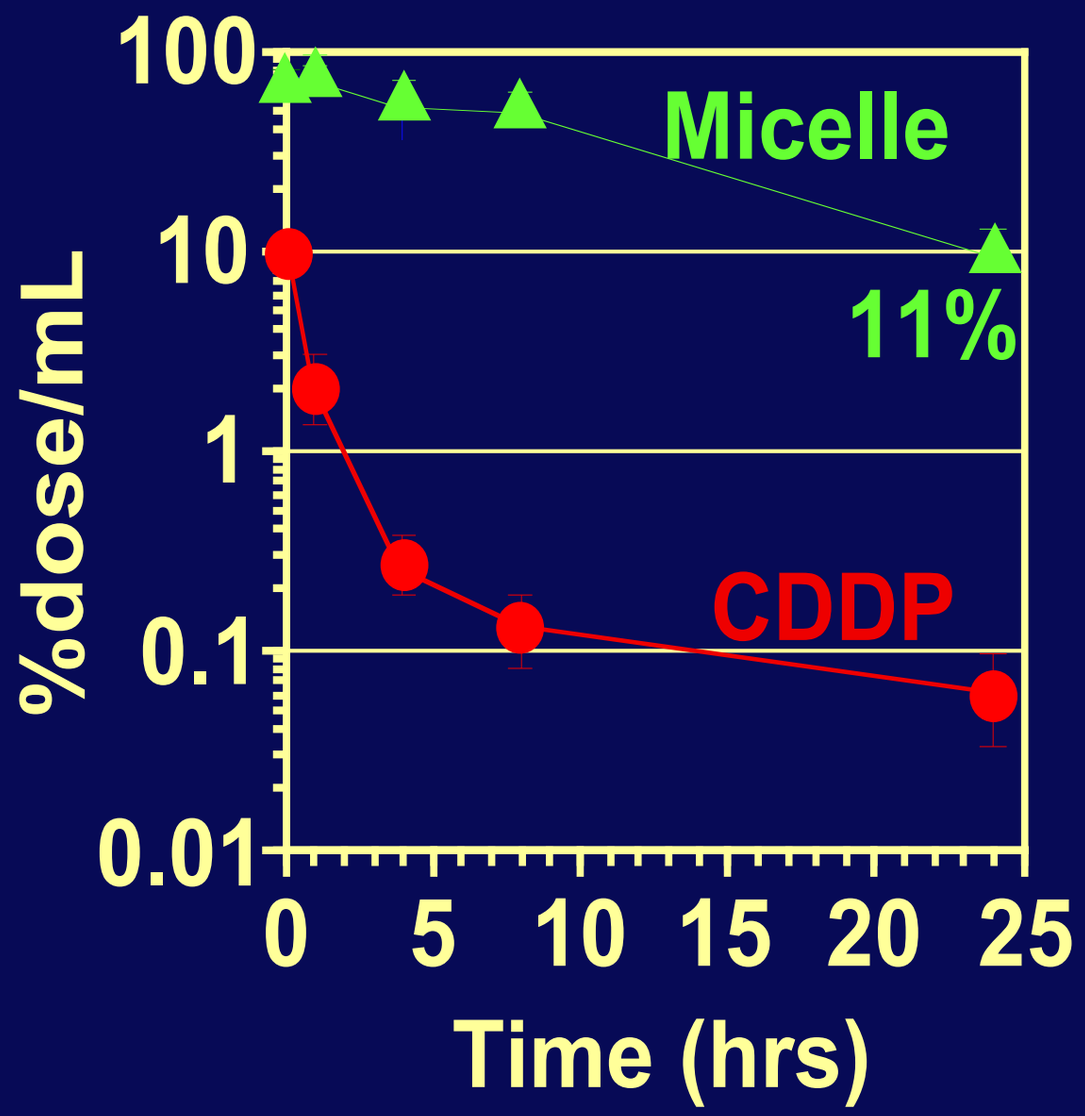
CDDP

self-association
(in water at 37˚C)

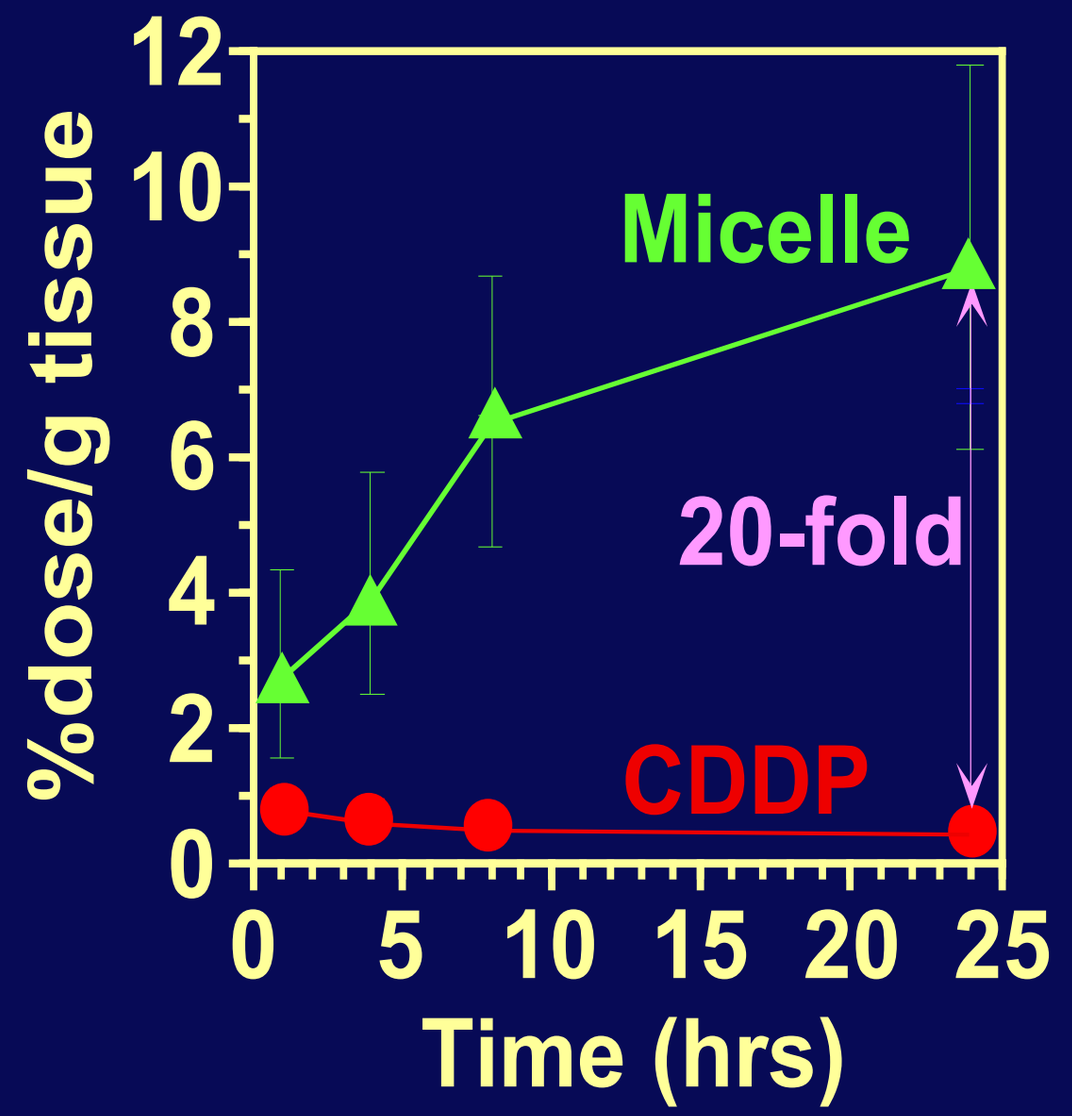
N. Nishiyama, et al, *Langmuir* 15(12), 4208-4212 (1999)

Biodistribution of CDDP-incorporated micelles

Plasma Pt concentration



Tumor accumulation

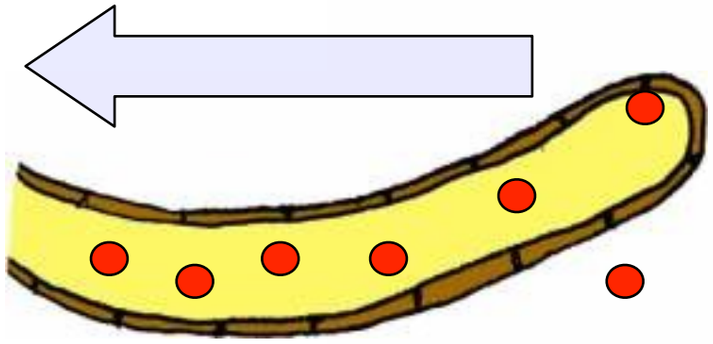


CDDP and CDDP-incorporated micelles were administered I.v. to Lewis lung carcinoma (LLC)-bearing C57BL6N mice (male, 6 week old, n=4)

Enhanced Permeability and Retention(EPR) Effect

Normal cell

Being ejected



The lymphatic system

Underdeveloped
lymphatic system

No way out

leaky vessel wall

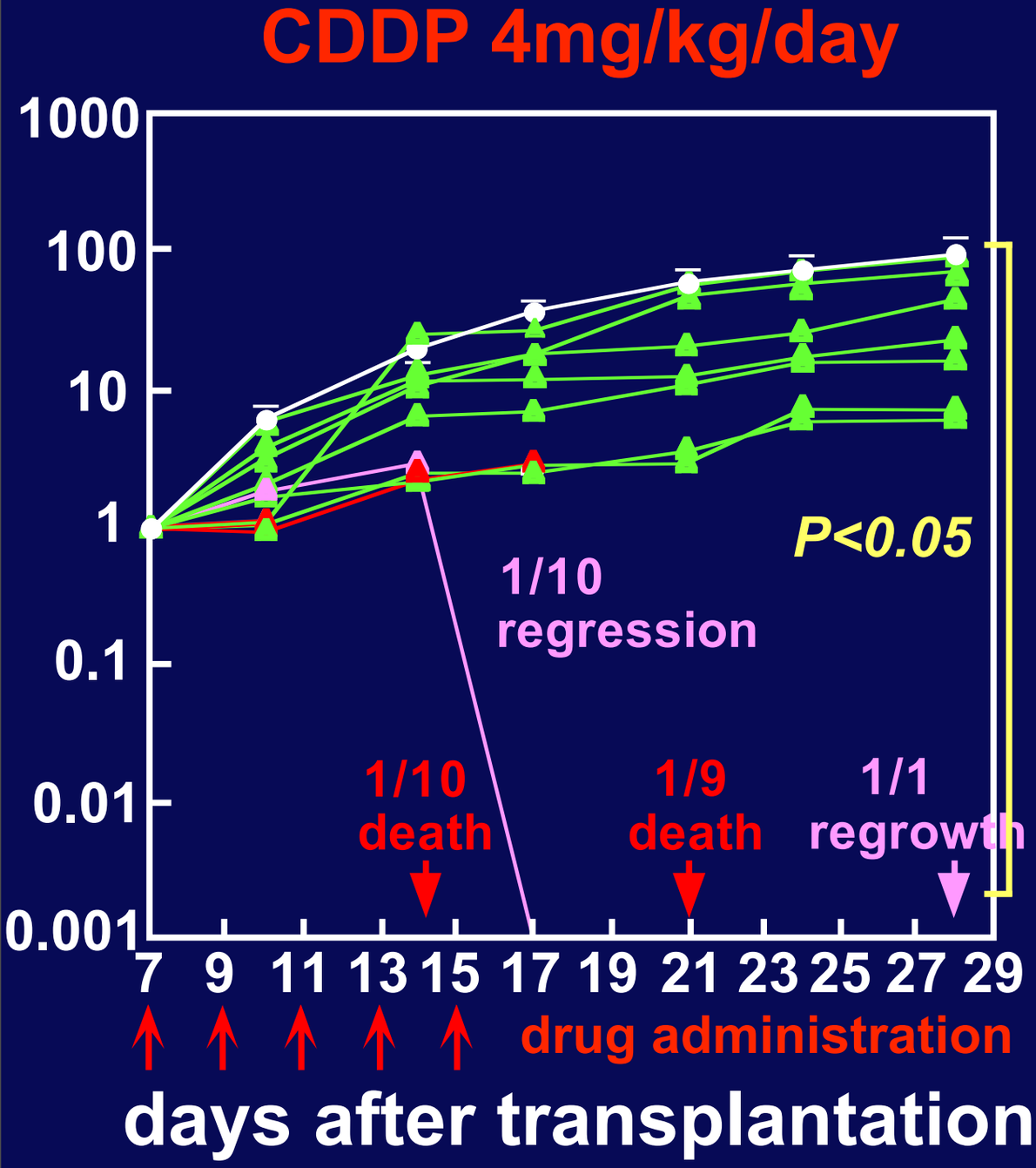
: Small drugs

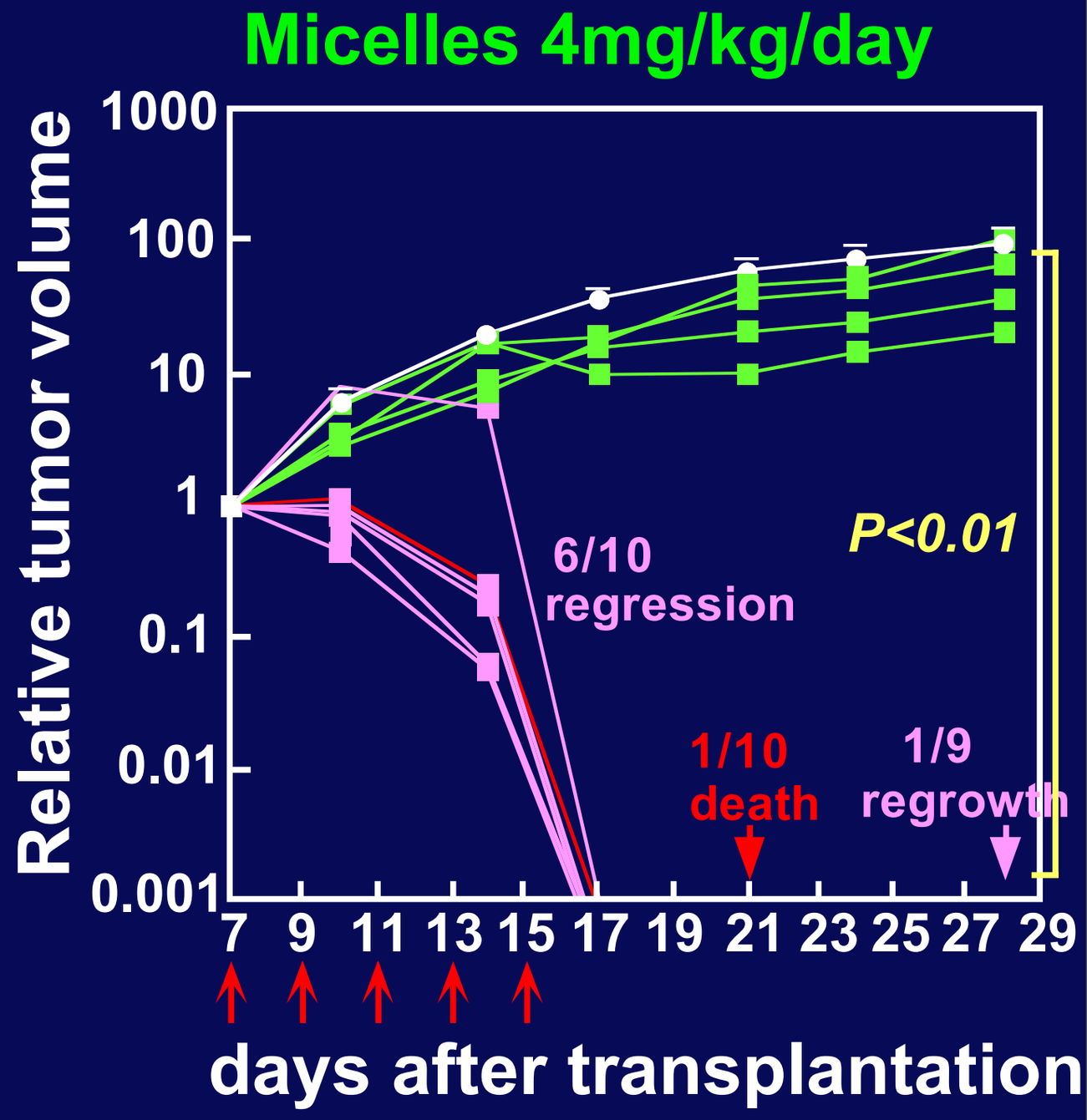
: The polymeric micelles

Cancerous cell

Anti-tumor activity of CDDP-incorporated micelles

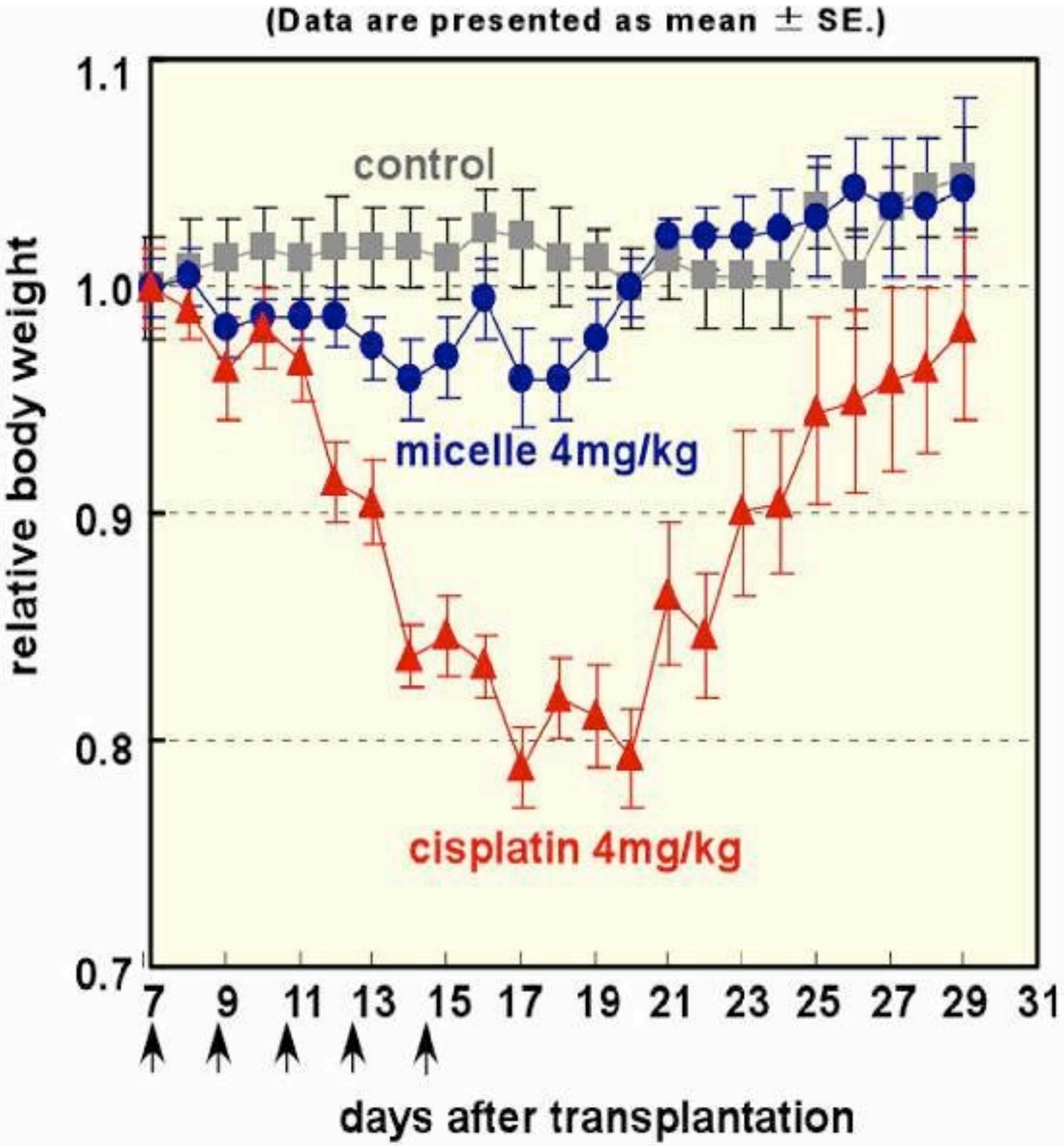
Colon 26-bearing CDF_1 mice (female, 6 week old, n=10) were treated 5 times with CDDP and CDDP-incorporated micelle (4mg/kg/day).





N. Nishiyama, et al, *Cancer Research*, 63, 8977-8983 (2003)

Change in body weight of mice treated with cisplatin and PEG-P(Glu(CDDP)) (12-60) micelles



Decrease in body weight	
cisplatin	20%
micelle	5%

Disappearance of primary tumor	
control	0/10
cisplatin	0/8
micelle	4/9

PEG-P(Glu(CDDP)) **(12-60)** micelles achieved lower toxicity and higher antitumor activity than same dose of cisplatin.

Polymeric Micelles from PEG-poly(amino acid) Block Copolymers in Clinical Development

Adriamycin (NK911:Nippon Kayaku Co.) :

Phase II Clinical Trial

Paclitaxel (NK105:NanoCarrier Co./Nippon Kayaku Co.) :

Phase II Clinical Trial

Cisplatin (NC6004:NanoCarrier Co.) :

Phase I/II Clinical Trial

Camptothecine derivative (SN-38) (NK012:Nippon Kayaku Co.) :

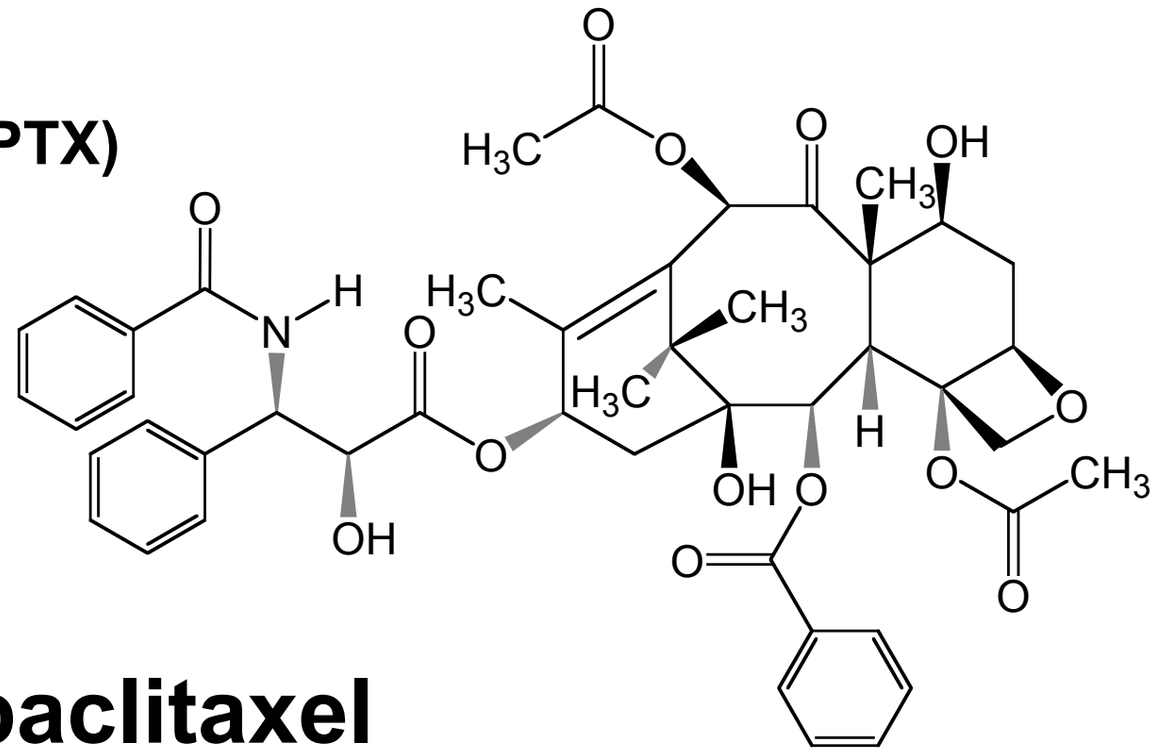
Phase I Clinical Trial

Dachplatin (NC4016/ND-1:NanoCarrier Co./Debio Pharm Co.) :

Phase I Clinical Trial

Preparation of Paclitaxel-loaded Polymeric Micelle (NK105)

Structure of paclitaxel (PTX)



Preparation of the micellar paclitaxel

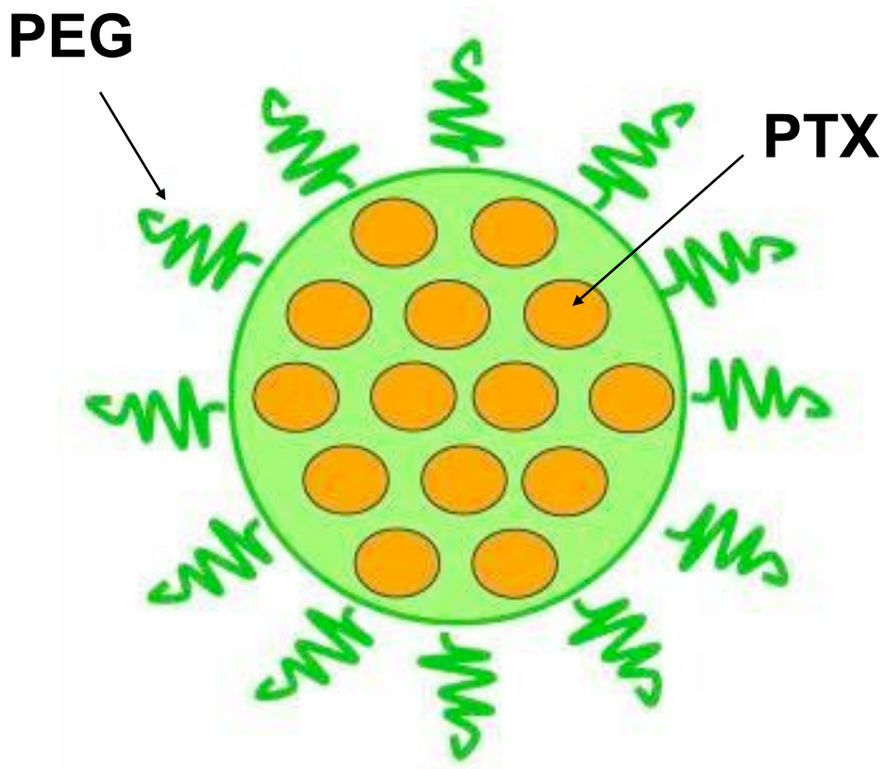
PEG-Hydrophobically modified poly(amino acid) block copolymer

+ (Mixed in dichloromethane)

paclitaxel

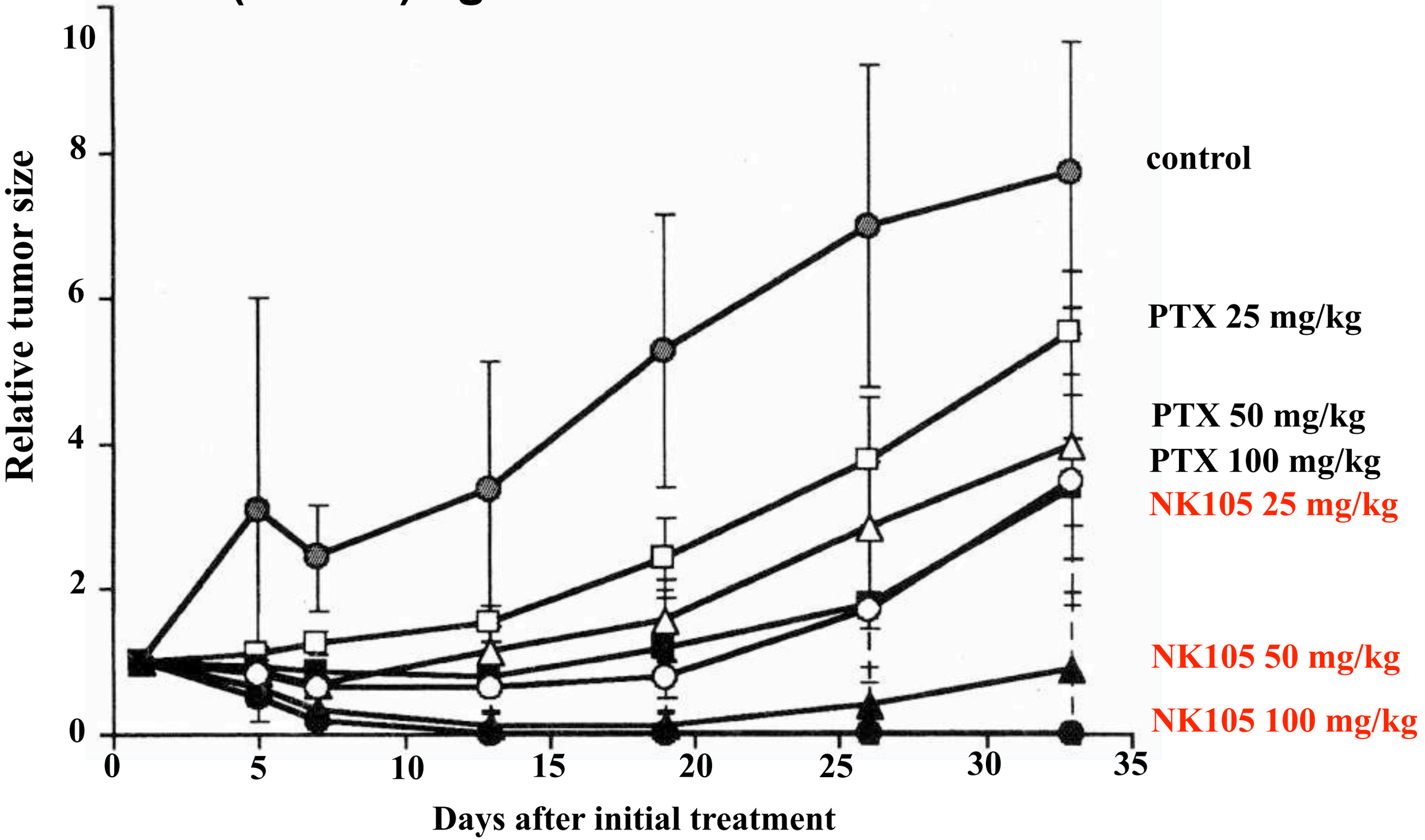
↓ **Emulsification**
Evaporation

Micellar paclitaxel (NK105)



T. Hamaguchi, et al, Br. J. Cancer, 92, 1240-1246 (2005)

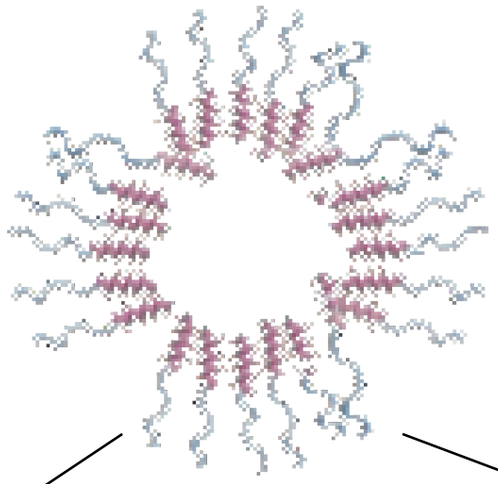
Anti-tumor activity of paclitaxel-loaded polymeric micelles (NK105) against HT-29 tumor in nude mice

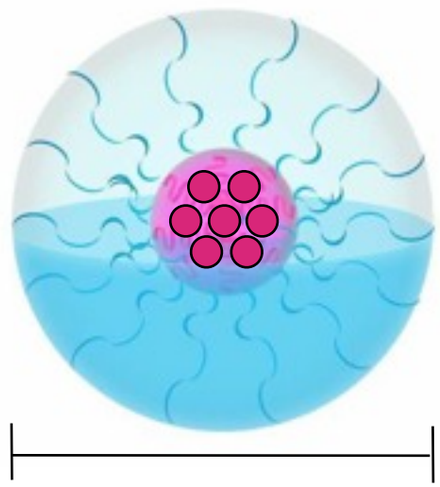


PTX (open) and NK105 (closed) were injected intravenously once weekly for 3 weeks at PTX-equivalent doses of 25, 50, and 100 mg/kg.

T. Hamaguchi, et al, *Br. J. Cancer*, 92, 1240-1246 (2005)

The pH-sensitive polymeric micelles





~60 nm

pH sensitive linker

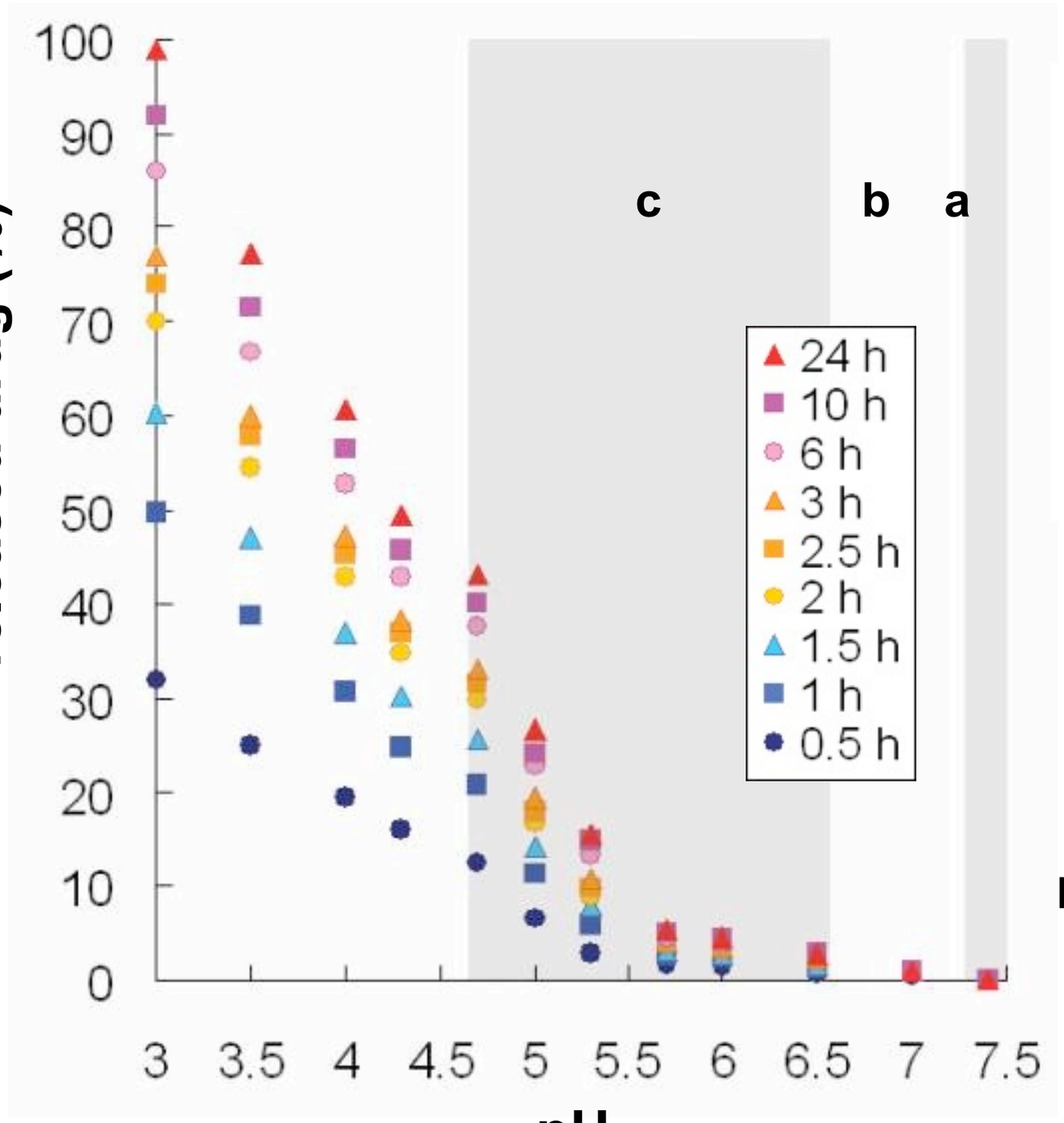
$[H^+]$

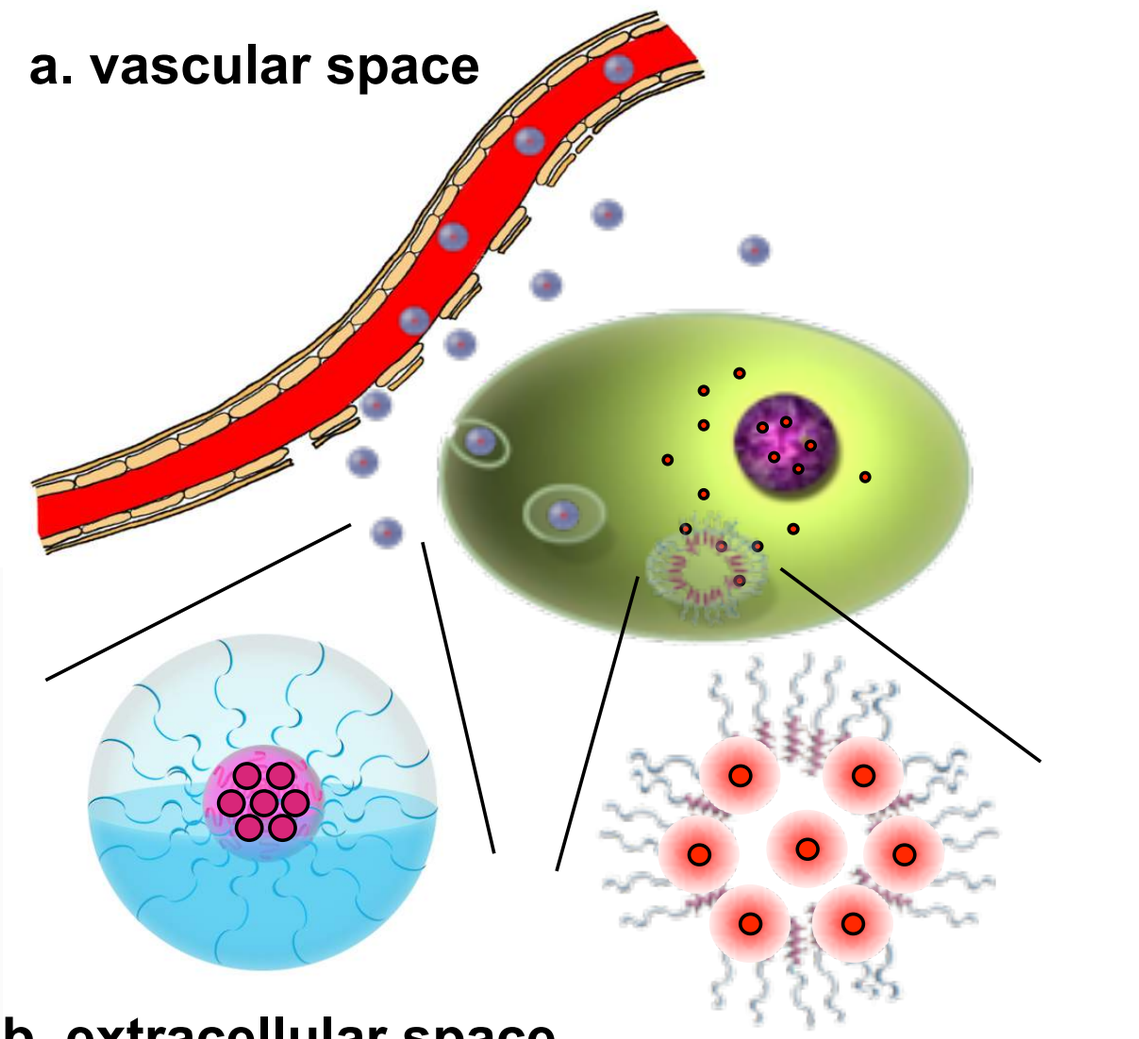
cleavable in acidic conditions (pH 5-6)

Adriamycin (ADR)

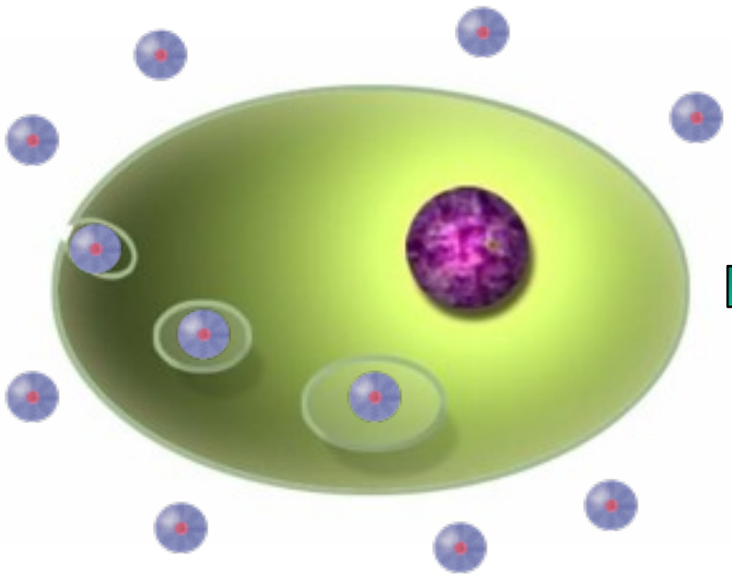
Y. S. Bae et al, *Angew. Chem., Int'l Ed.* 42, 4640 (2003); *Bioconj. Chem.* 16, 122 (2005); *Bioconj. Chem.* 18, 1131 (2007)

pH-sensitivity adjusted to intracellular endosomal space

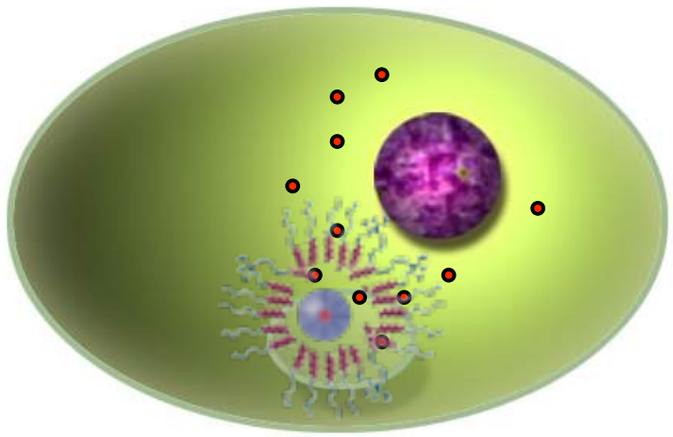




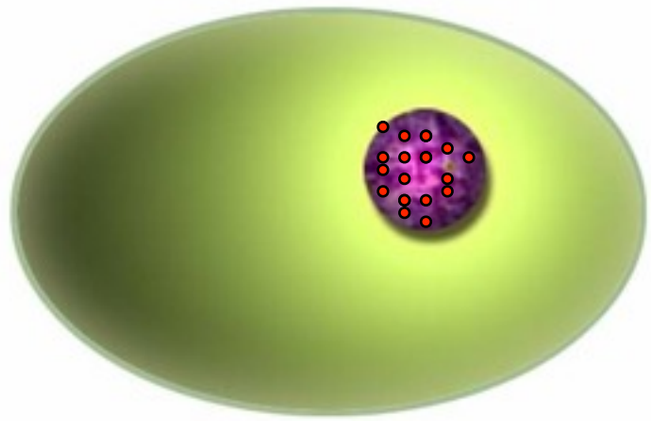
Observation of intracellular drug release by fluorescence



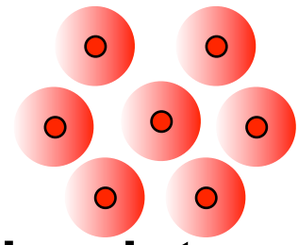
FL remains quenched as long as the micelles are stable



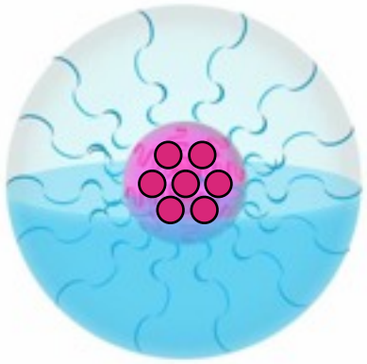
Intracellular localization and drug release of the micelles are detectable



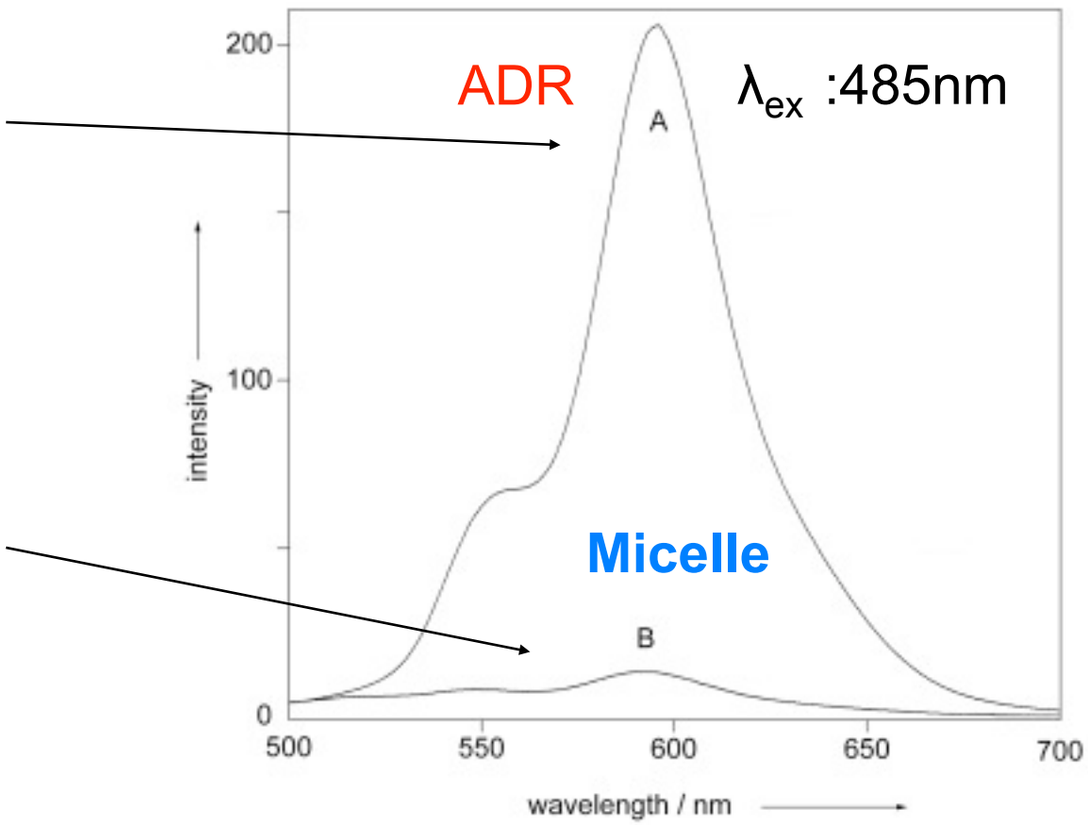
We can expect fate of released drugs in the cell

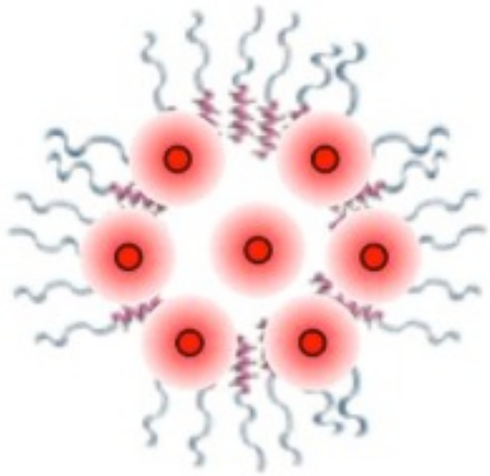


ADR has intense FL



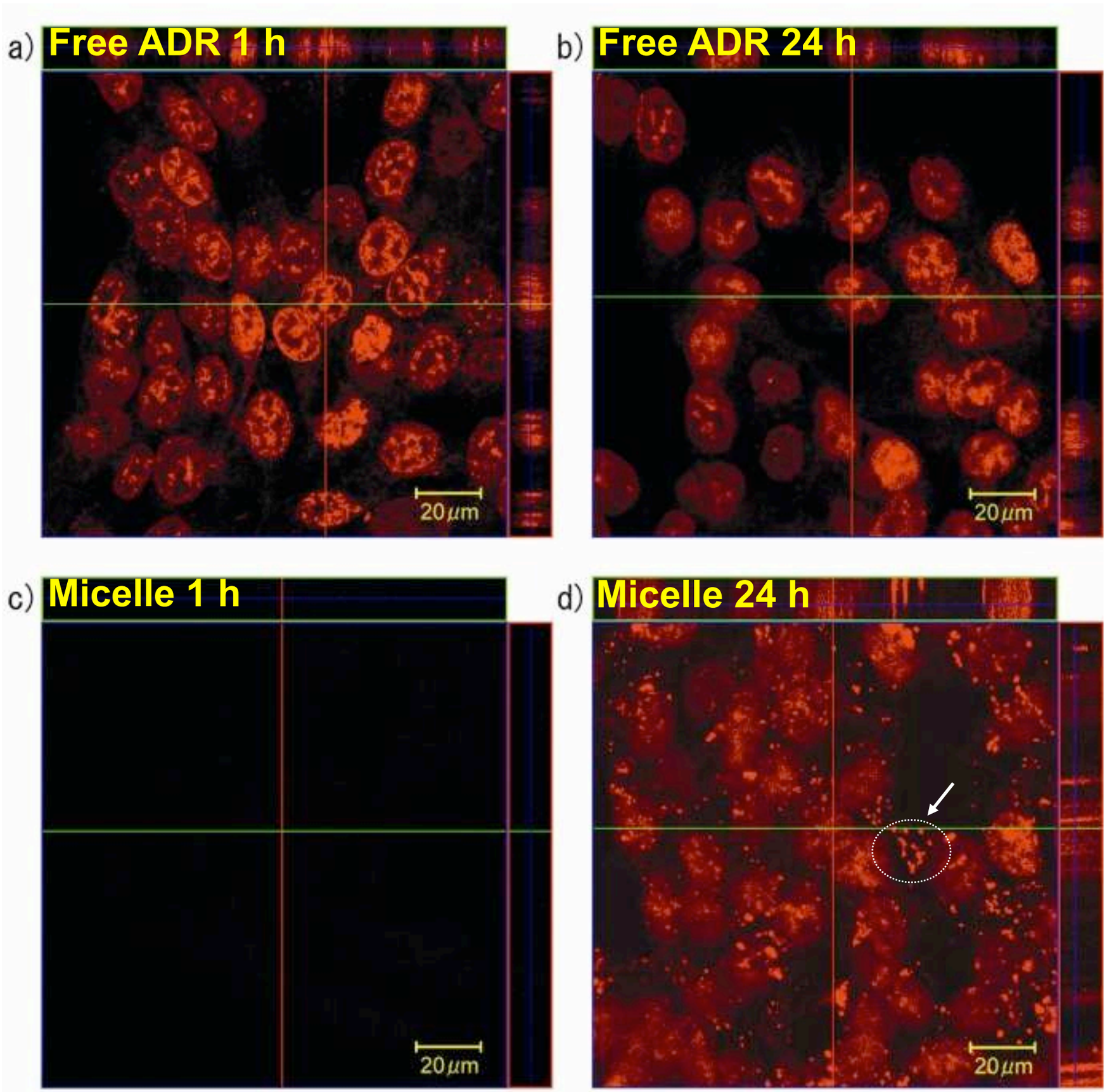
FL is quenched in the micelle core





FL becomes detectable with drug release

Intracellular distribution of ADR



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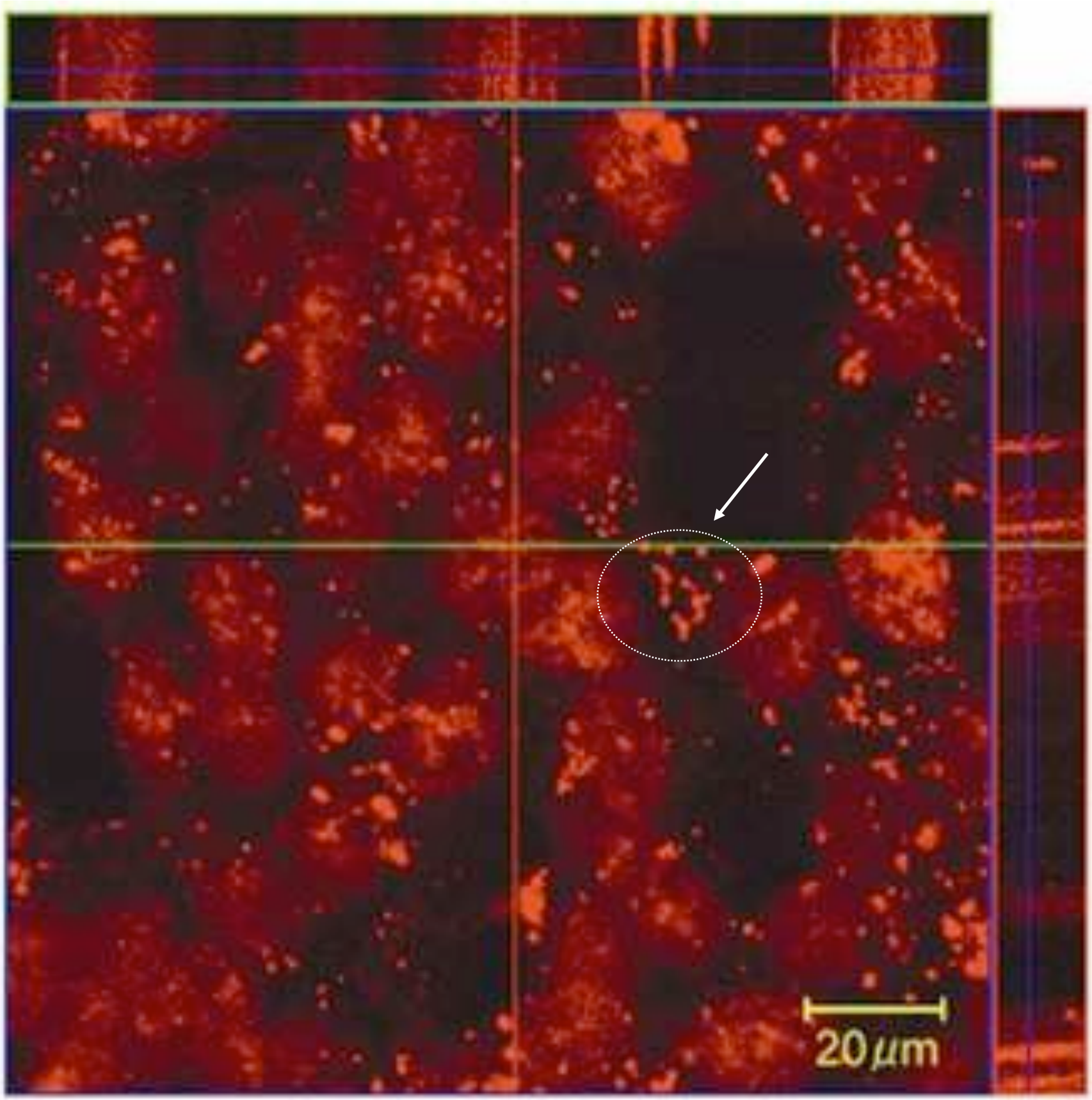
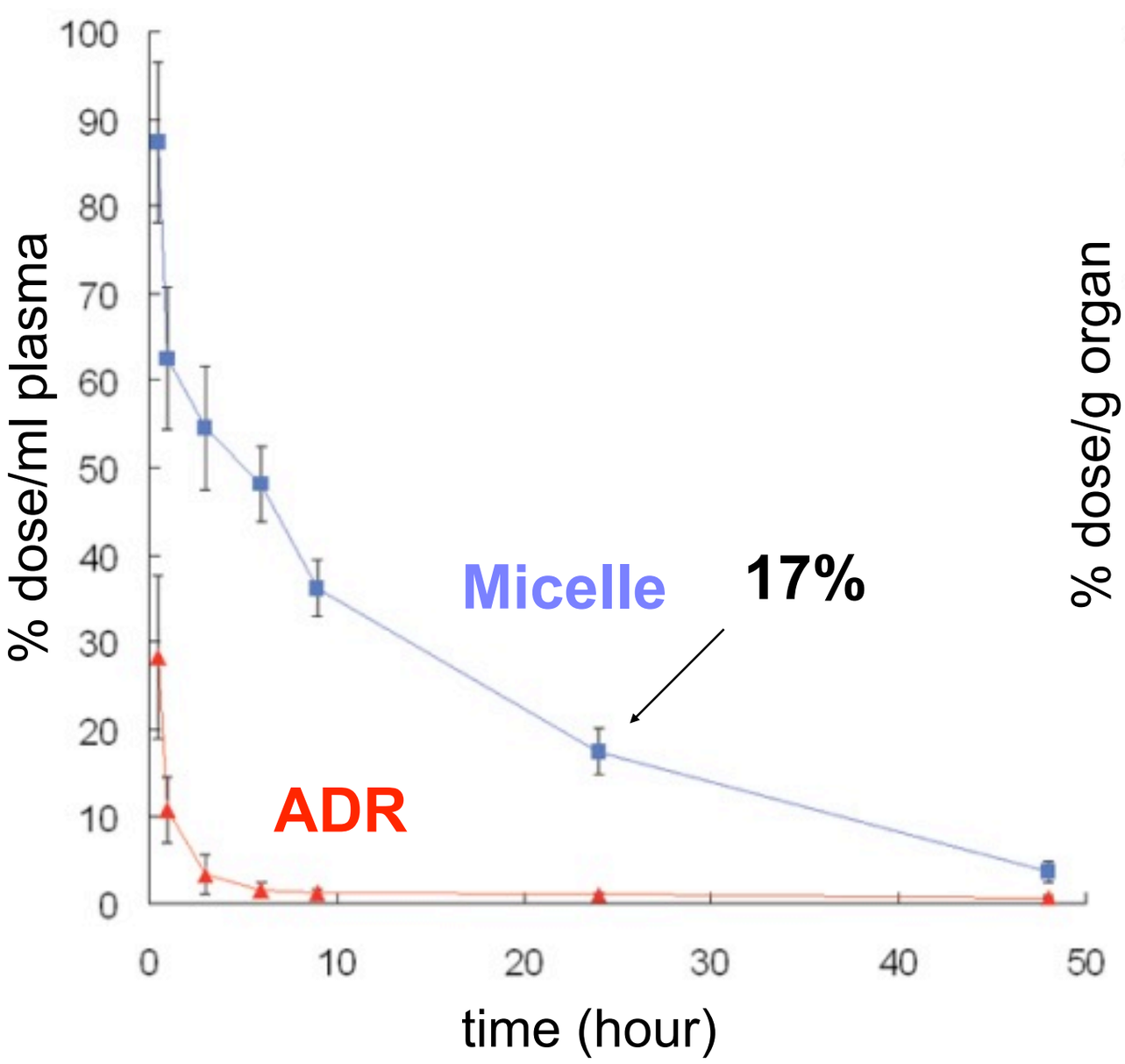
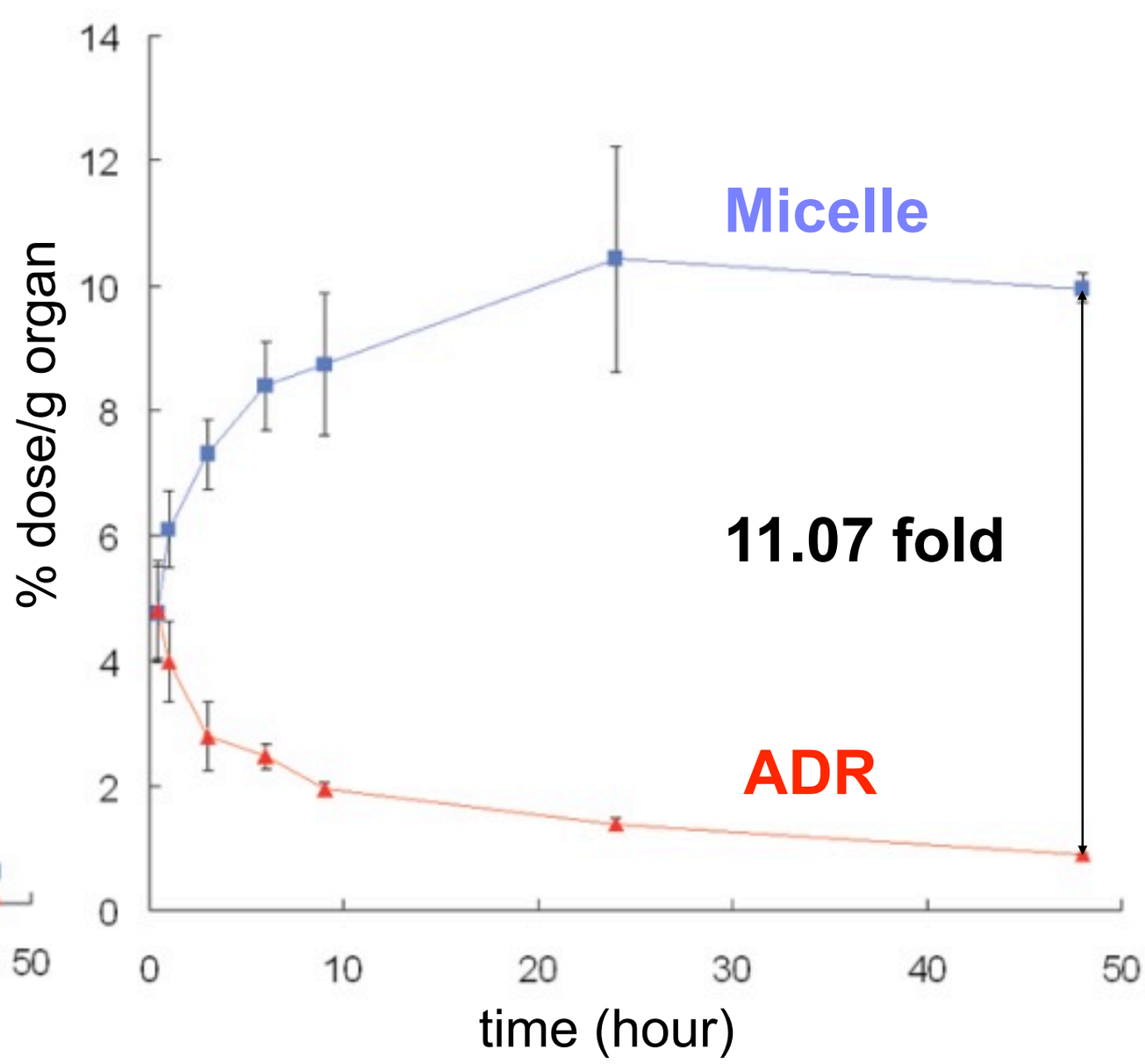


Biodistribution of free and micellar ADR



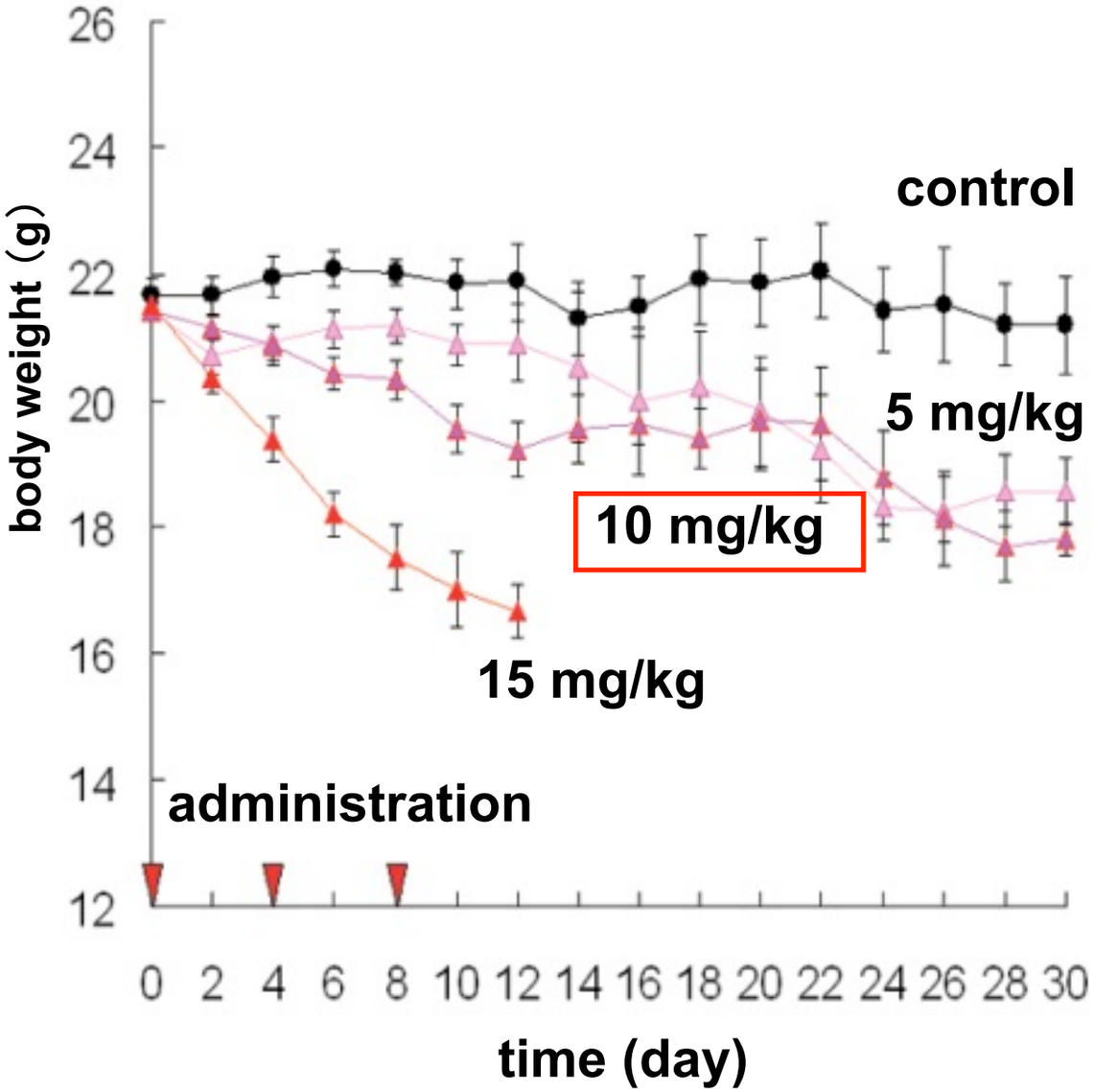


Prolonged circulation and tumor specific accumulation of micellar ADR

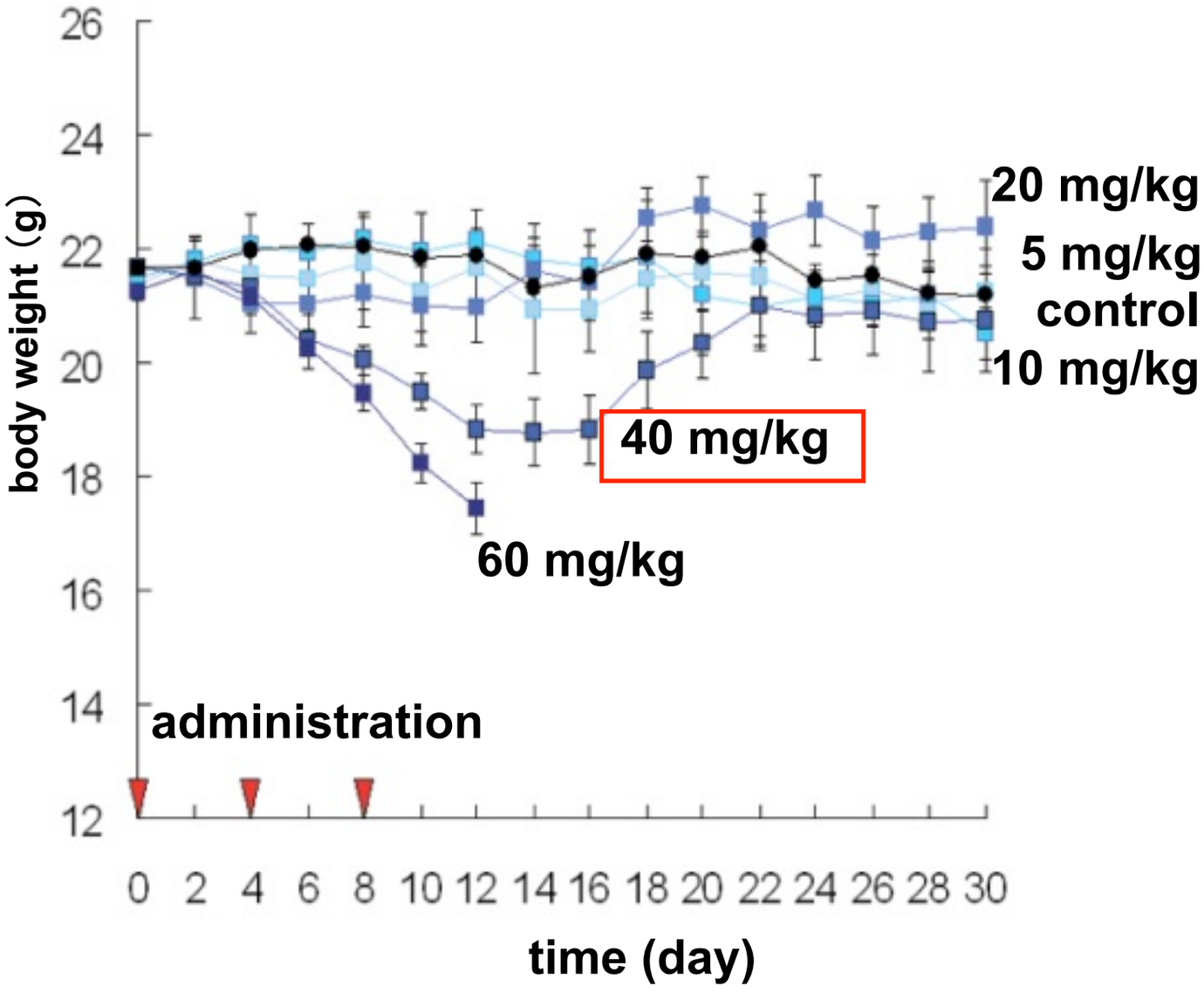
Y. S. Bae, et al, *Bioconj. Chem.*, 16, 122-130 (2005)

In vivo toxicity (body weight change) of free-ADR and micellar-ADR

Free-ADR



Micellar-ADR



Micellar-ADR exhibited more than 4 times higher MTD compared to free-ADR

In vivo antitumor activity

sample	dose (mg/kg) [a]	body weight change on day 30 (%) [b]	toxic death	duration days of tumor growth [c]	complete cure
control	0	-2.18±1.74	0/6	3.74	0/6
ADR	5	-13.35±0.59	0/6	4.21	0/6
	10	-16.84±1.26	0/6	14.59	1/6
	15	–	6/6	–	–
Micelles	5	-0.89±1.68	0/6	3.88	0/6
	10	-4.51±1.44	0/6	3.97	0/6
	20	3.13±1.60	0/6	22.05	2/6
	40	-4.07±0.92	0/6	27.83	3/6
	60	–	6/6	–	–

[a]Administrations were carried out three times with a 4-day interval, and doses were determined in free ADR equivalents.

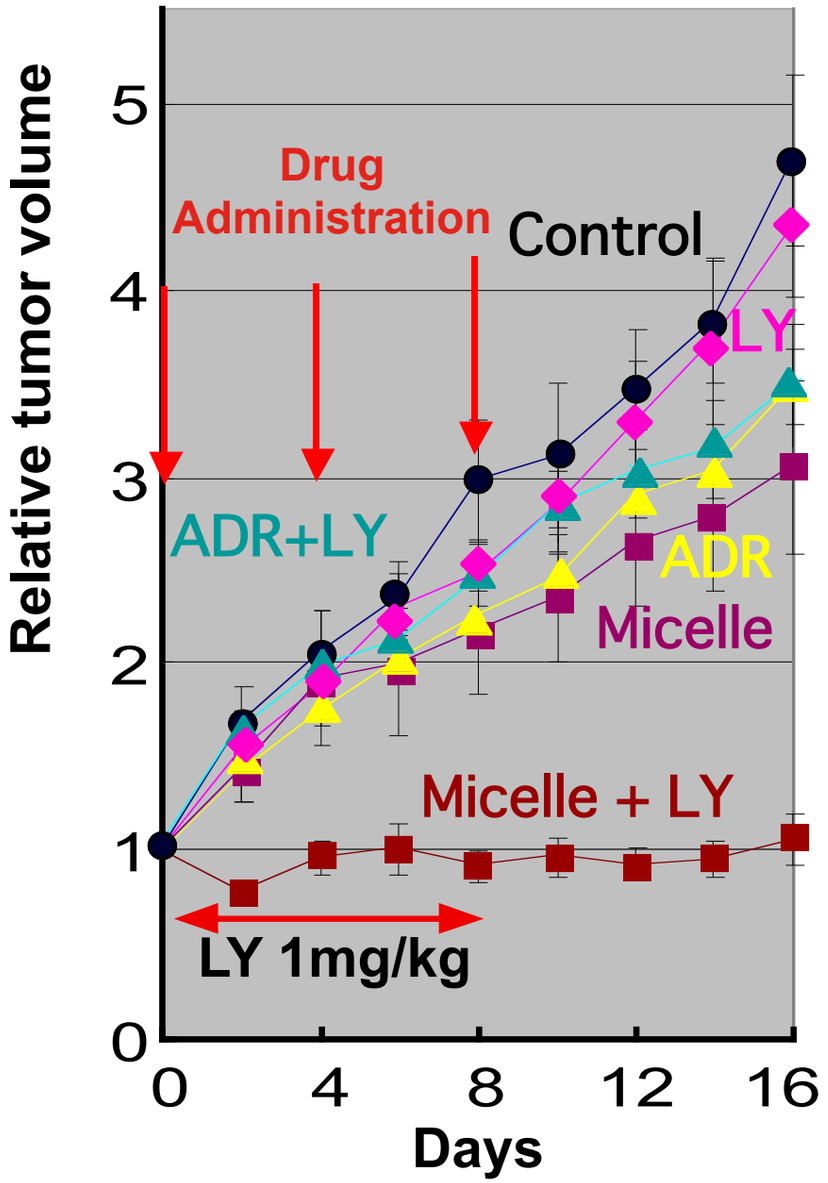
[b]Body weights were measured on day 30 after the first injection to compare long-term toxicity between ADR and the micelles. Values are expressed as mean±SEM.

[c]Duration to reach 5-fold initial tumor volume.

Y. S. Bae, et al, *Bioconj. Chem.*, 16, 122-130 (2005)

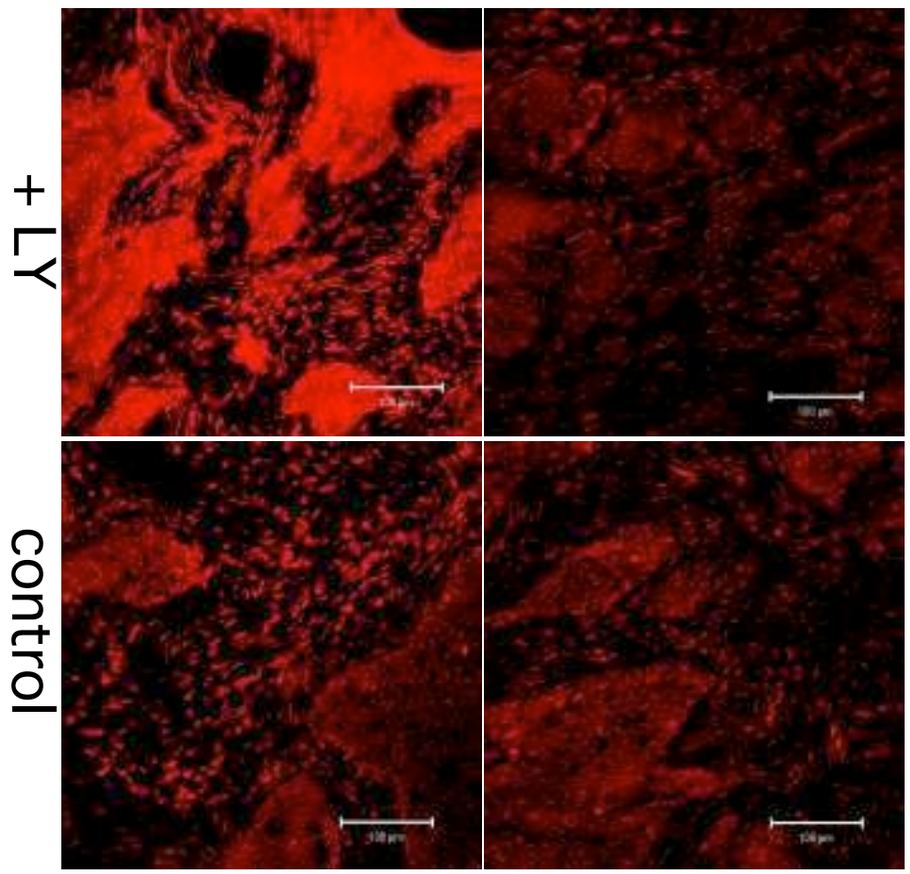
Treatment of intractable pancreatic cancer by pH-sensitive polymeric micelles

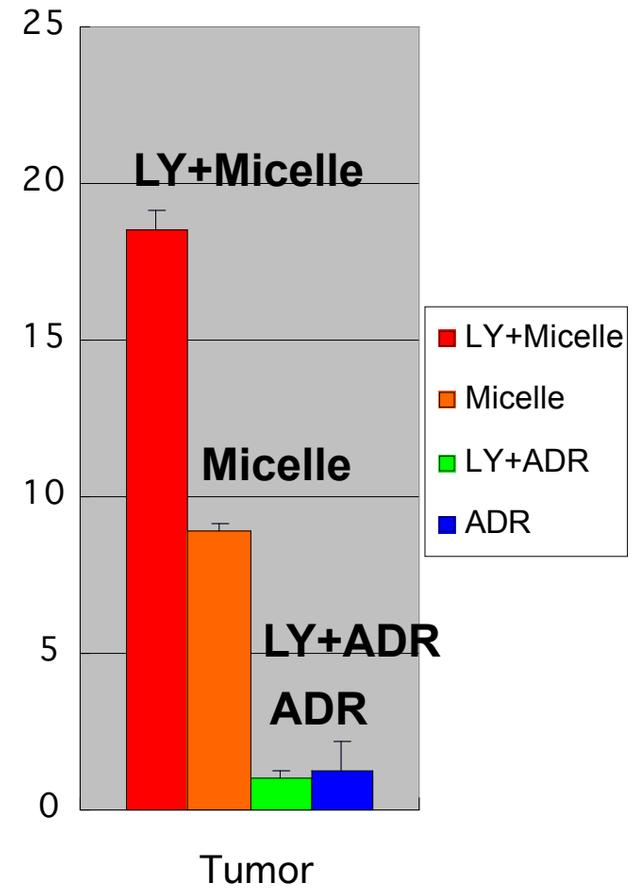
Human pancreatic cancer(BxPC3)
(characterized by hypovascularity and thick fibrosis)



LY: TGF-β inhibitor
(Reagent to transiently increase the permeability of tumor capillary)

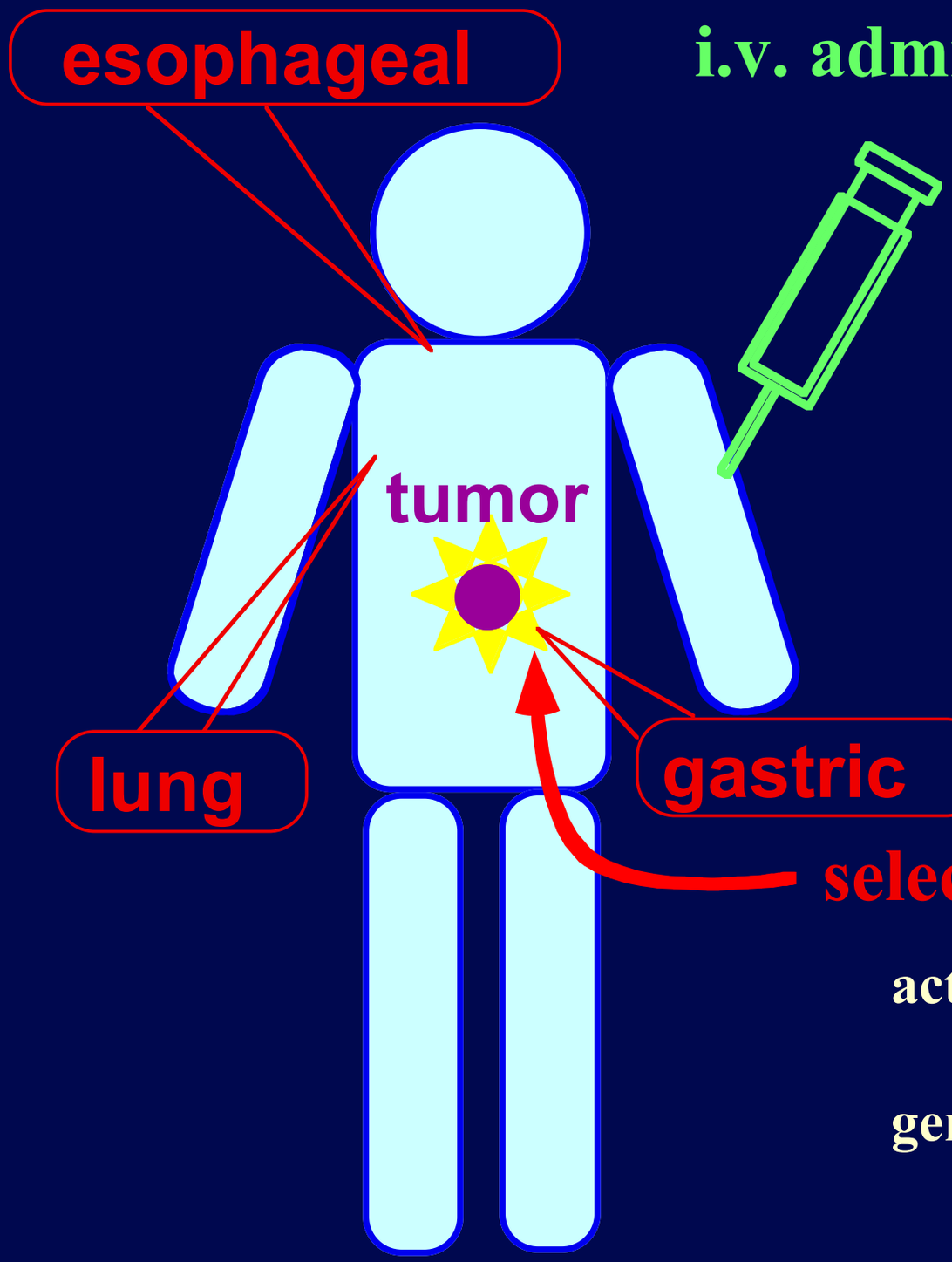
Accumulation of Micelle-ADR into BxPC3





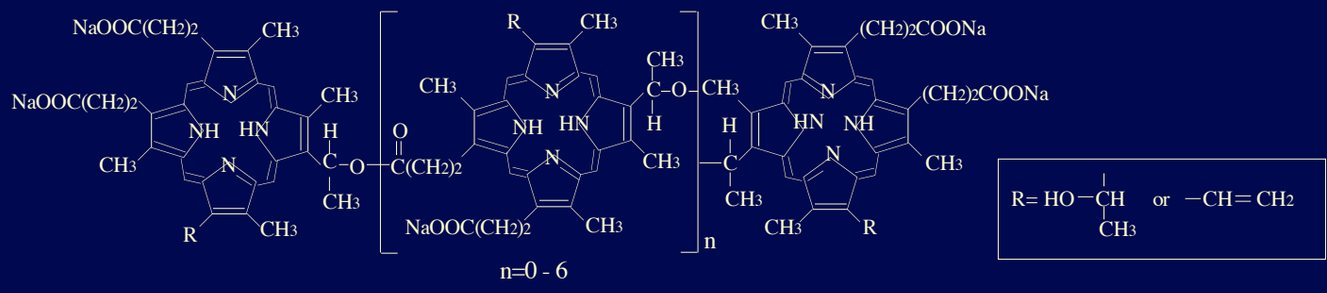
M. R. Kano, et al, *Proc. Nat'l. Acad. Sci., USA*, 104(9), 3460-3465 (2007)

Photodynamic therapy (PDT)



i.v. administration of photosensitizer

(A) Photofrin (PII)



n=0 - 6

(B) metallo-phthalocyanines (MePc)

selective irradiation of light

activation of photosensitizer

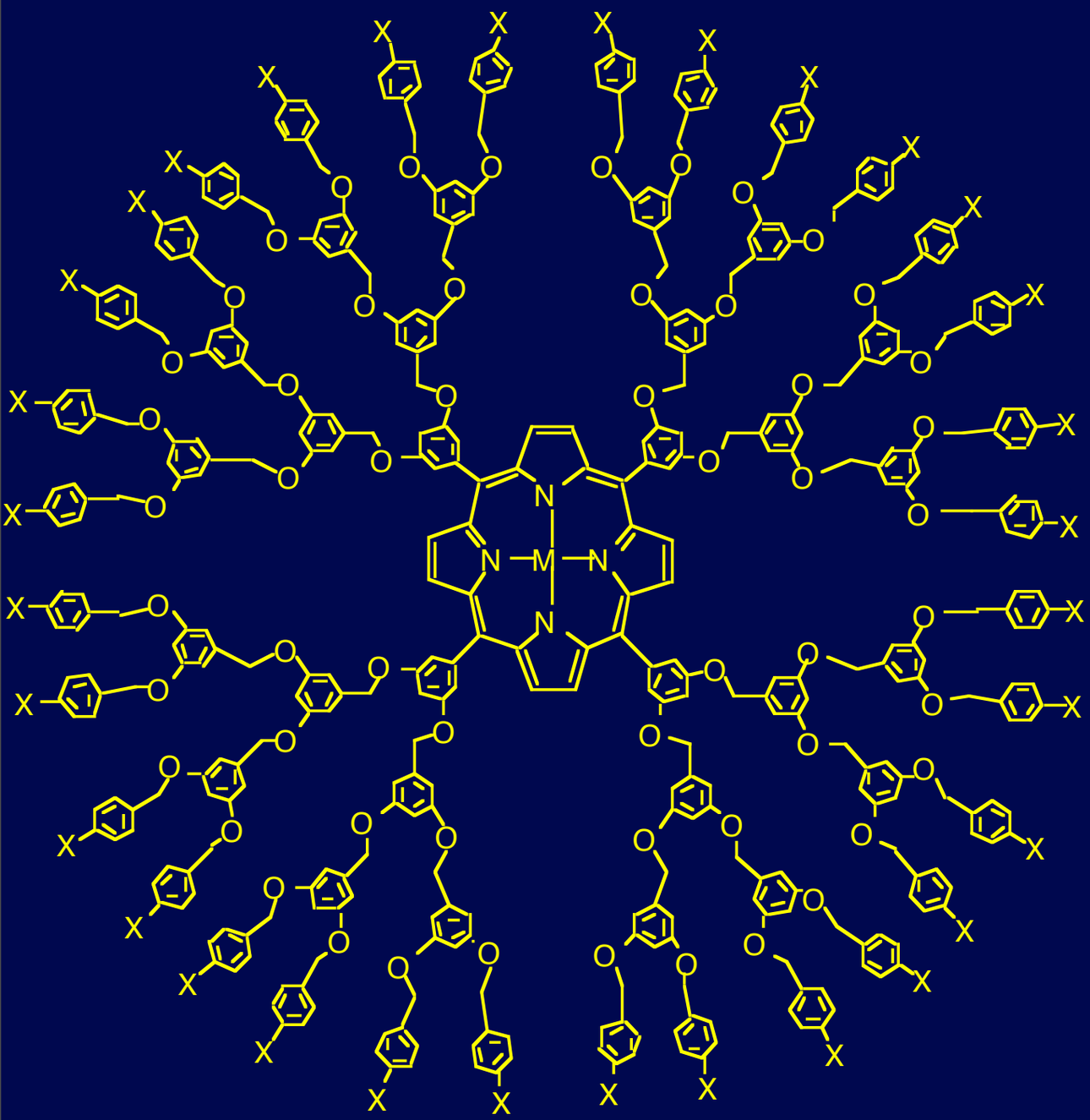
↓

generation of highly reactive oxygen species

↓

selective toxicity in tumor

Ionic dendrimer porphyrins (DP) as a novel type of photosensitizer



- **P32(+)DPZn:**

  X=$CONH(CH_2)_2NMe_3^+Cl^-$ 1)

  or $CONH(CH_2)_2NH_3^+Cl^-$ 2)

  M=Zn

- **P32(-)DPZn:**

  X=COO^-K^+ 1)

  M=Zn

1) Sadamoto, R.; Tomioka, N.; Aida, T. *J. Am. Chem. Soc.* 1996, *118*, 3978-3979

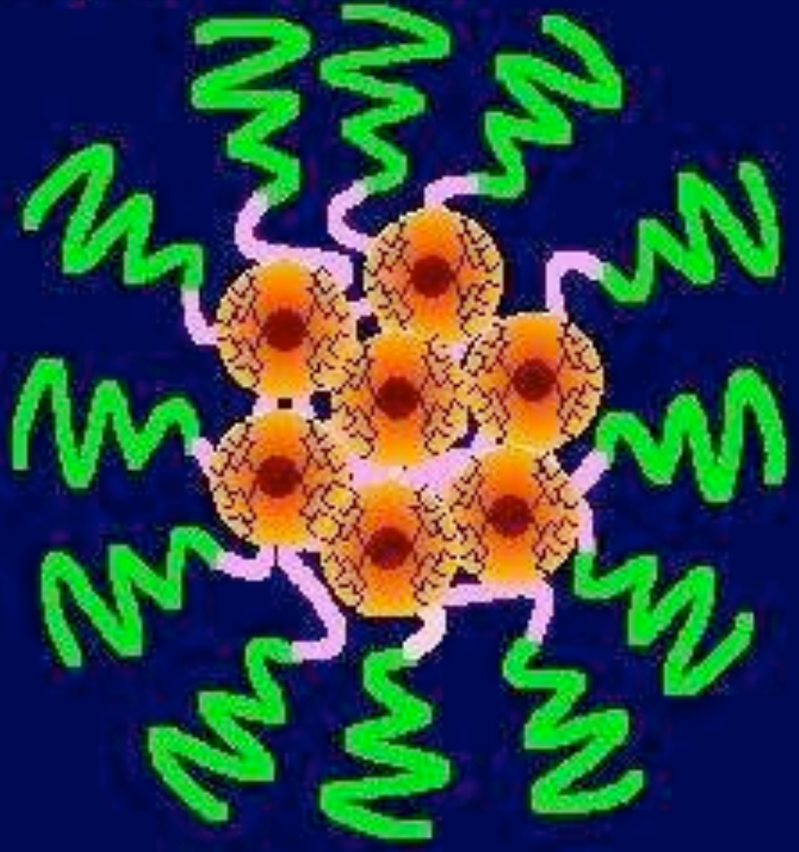
2) Zhang, G.; Kataoka, K.; et al, *Macromolecules*, 2003, *36*, 1304-1309

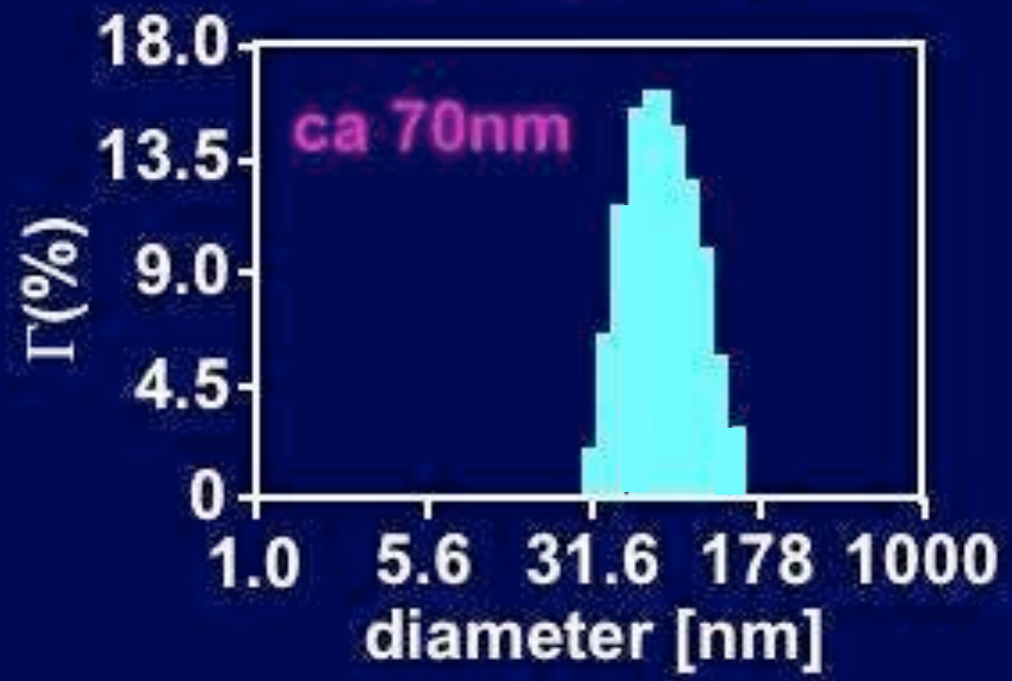
Polyion complex (PIC) micelles incorporating ionic dendrimer porphyrin (DP)

DP

PEG-P(Lys)

Self-assembly



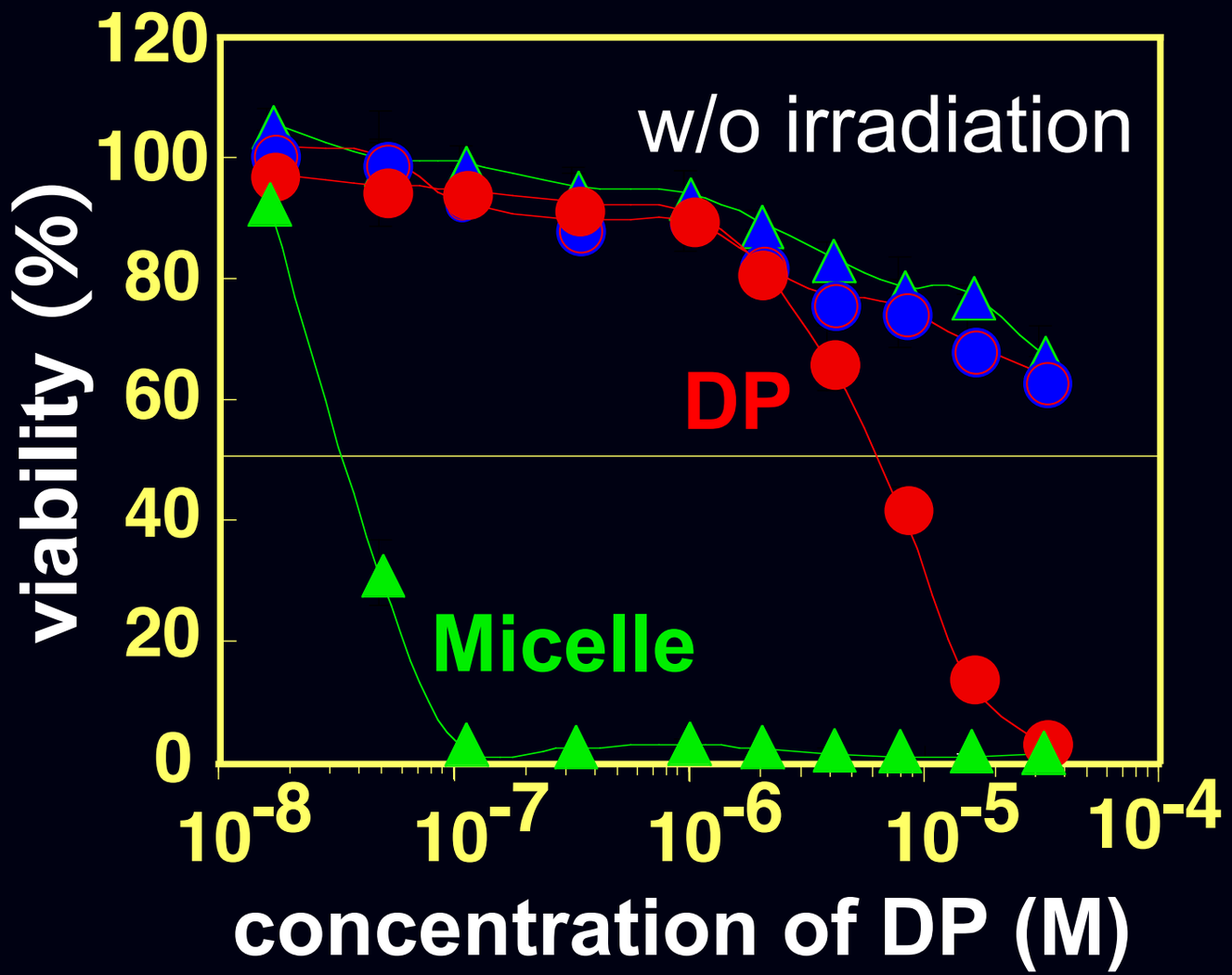


High stability against salt concentration (>300mM) and pH (6 - 8.5)

H. R. Stapert, et al, *Langmuir* 16, 8182 (2000); W. D. Jang, et al, *Angew. Chem., Int'l. Ed.*, 44, 419 (2005)

Photocytotoxicity of DP and DP-incorporated micelles

photocytotoxicity (LLC cells)



6hr incubation ⟶ wash with PBS ⟶ irradiation ⟶ 48hr incubation

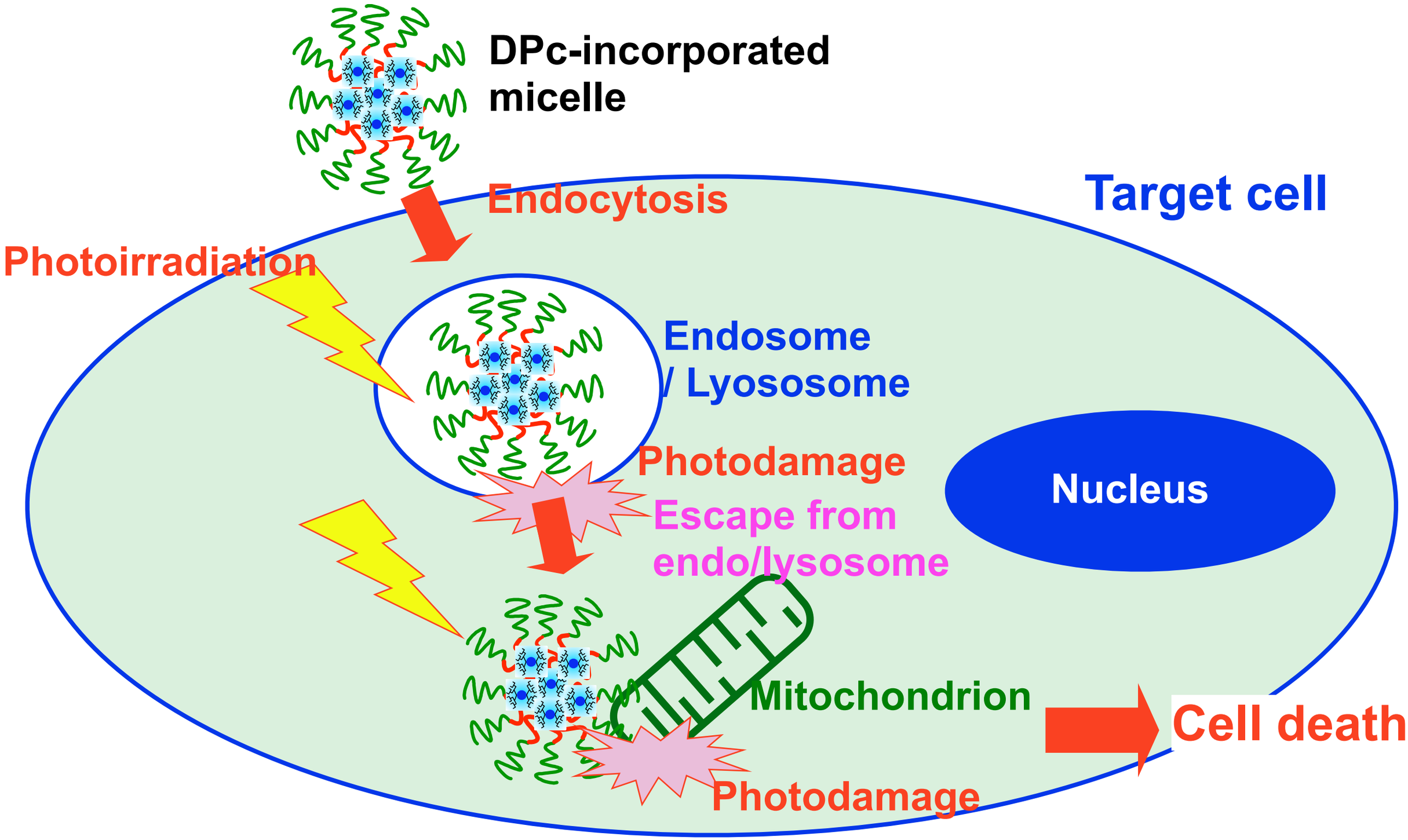
Incorporation of DP into the micelle achieved approximately 280-fold increase in photocyto-toxicity.

This result ensures safety after PDT, because the micelle is assumed to dissociate finally.

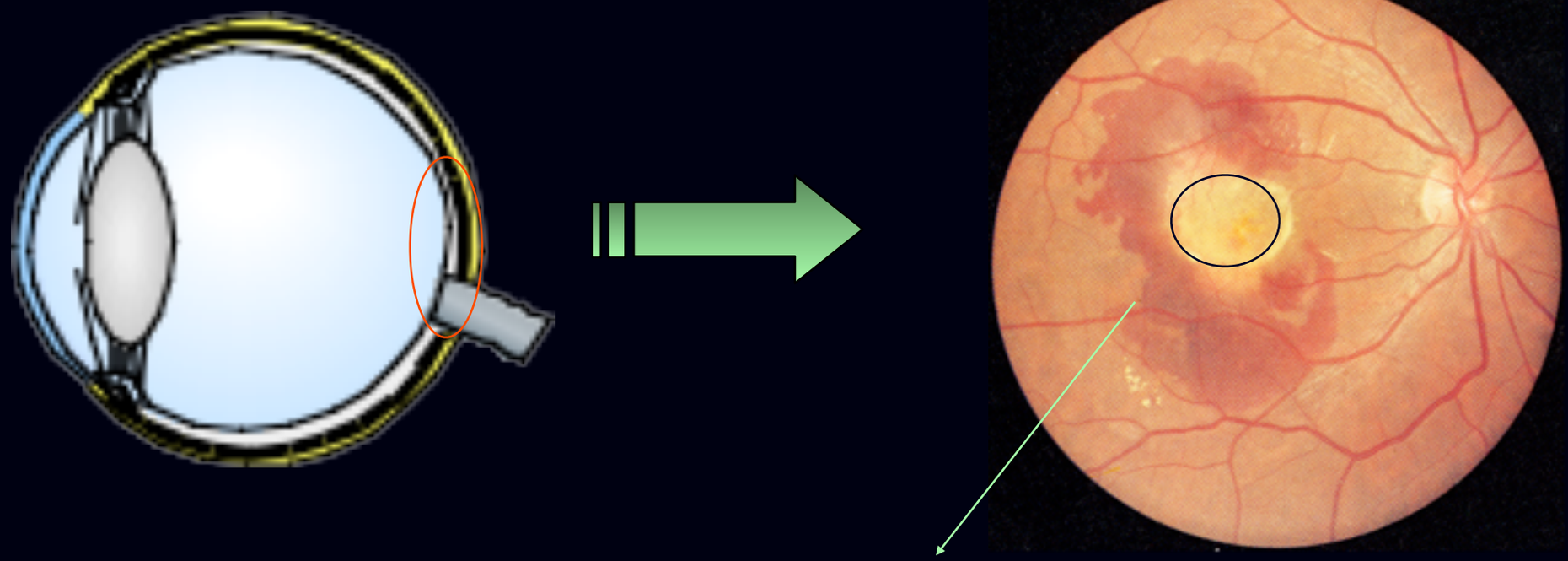
W. D. Jang, et al, *Angew. Chem., Int'l. Ed.*, 44, 419 (2005)

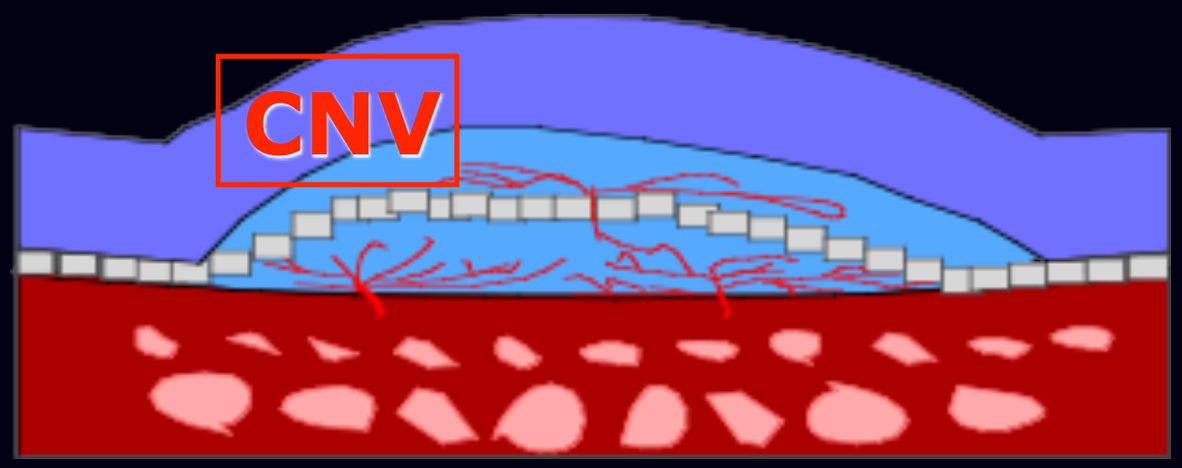
Hypothetic Mechanism of Cell Death

endocytic uptake → photochemical disruption of endosomal membrane → endosomal escape → interactions with mitochondrial membranes and their photochemical disruption



Exudative age-related macular degeneration (wet AMD) is characterized by choroidal neovascularization (CNV), and is a major cause of visual loss in developed countries.





% of CNV Occlusion

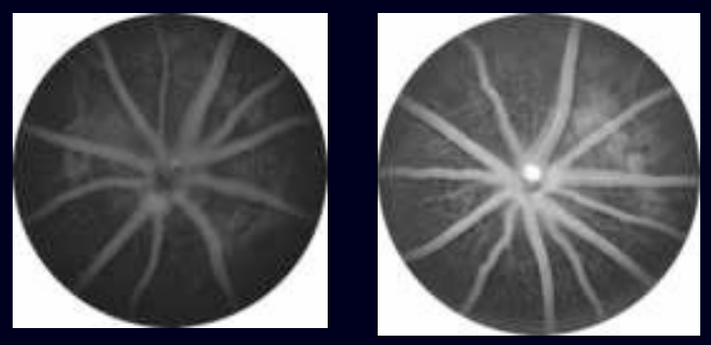
Laser Intensity (J/cm^2)	CNV Occlusion [%]			
	1 day after PDT		7 days after PDT	
	PIC micelle	Visudyne*	PIC micelle	Visudyne*
5	63.6	--	81.8	--
10	75.0	31	81.3	6
25	88.8	83	83.3	33
50	73.3	54	80.0	36
100	90.9	42	81.8	44

Zacks et al. IOVS, 2002

Fluorescent imaging of eyeground

Before PDT

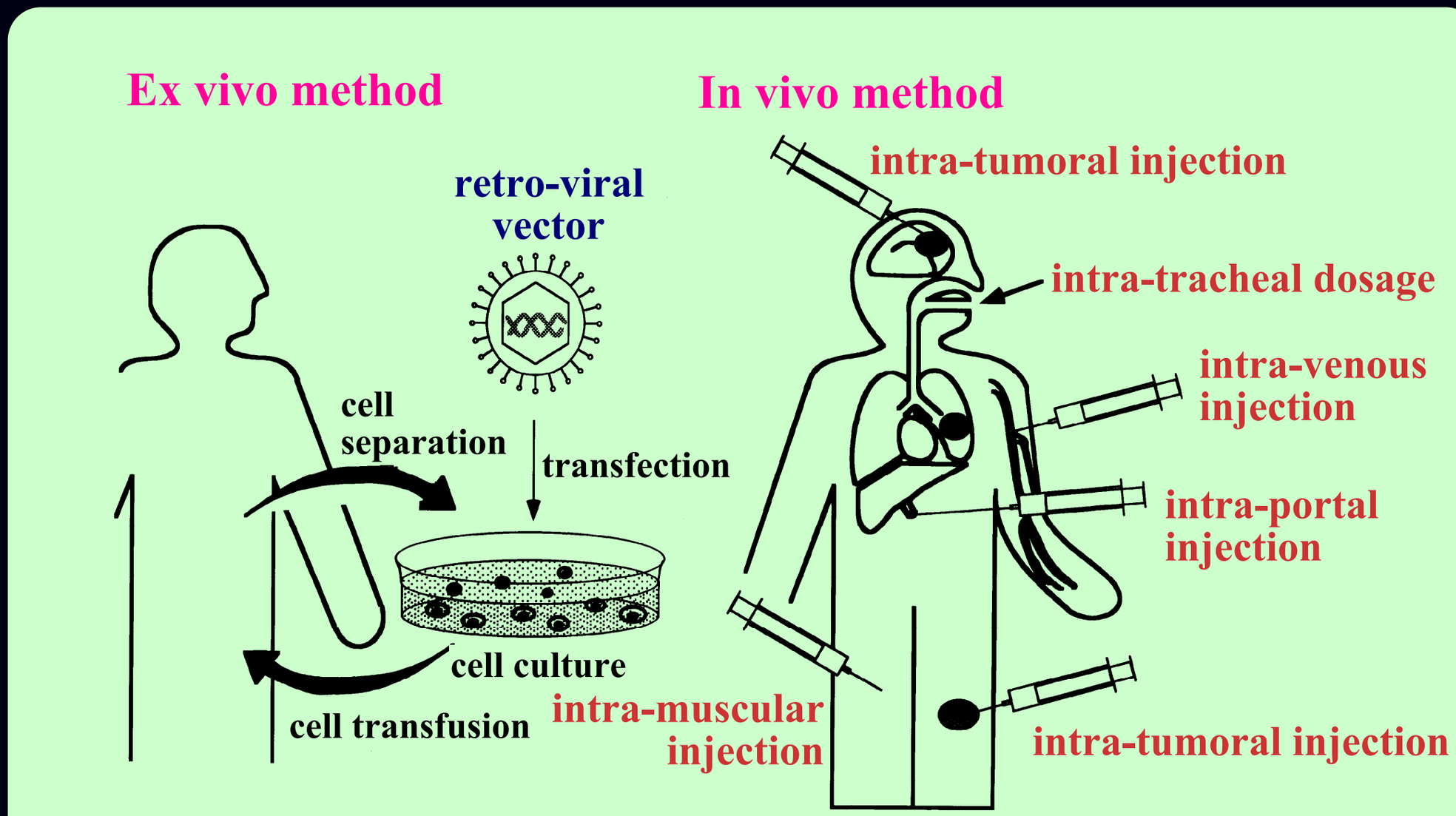
After PDT



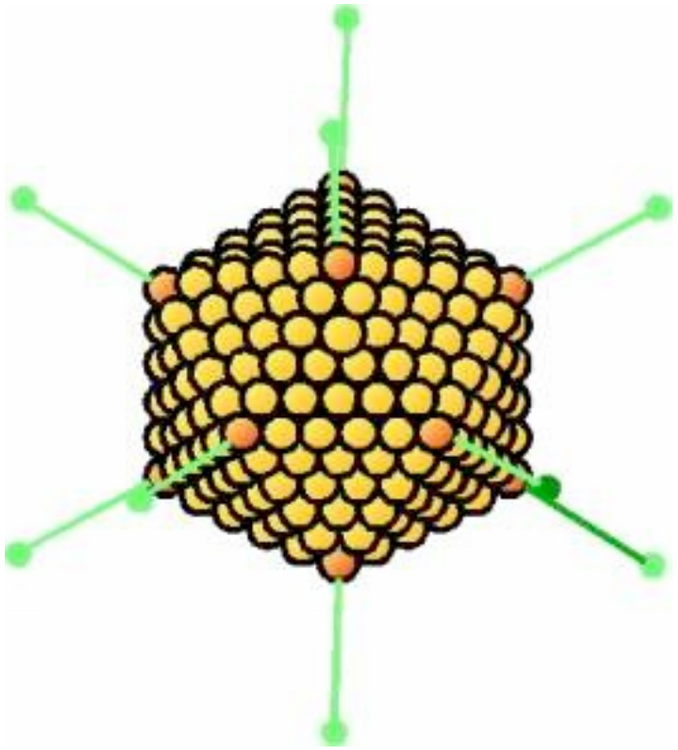
R. Ideta, et al, *Nano Lett.*, 5(12), 2426 (2005)

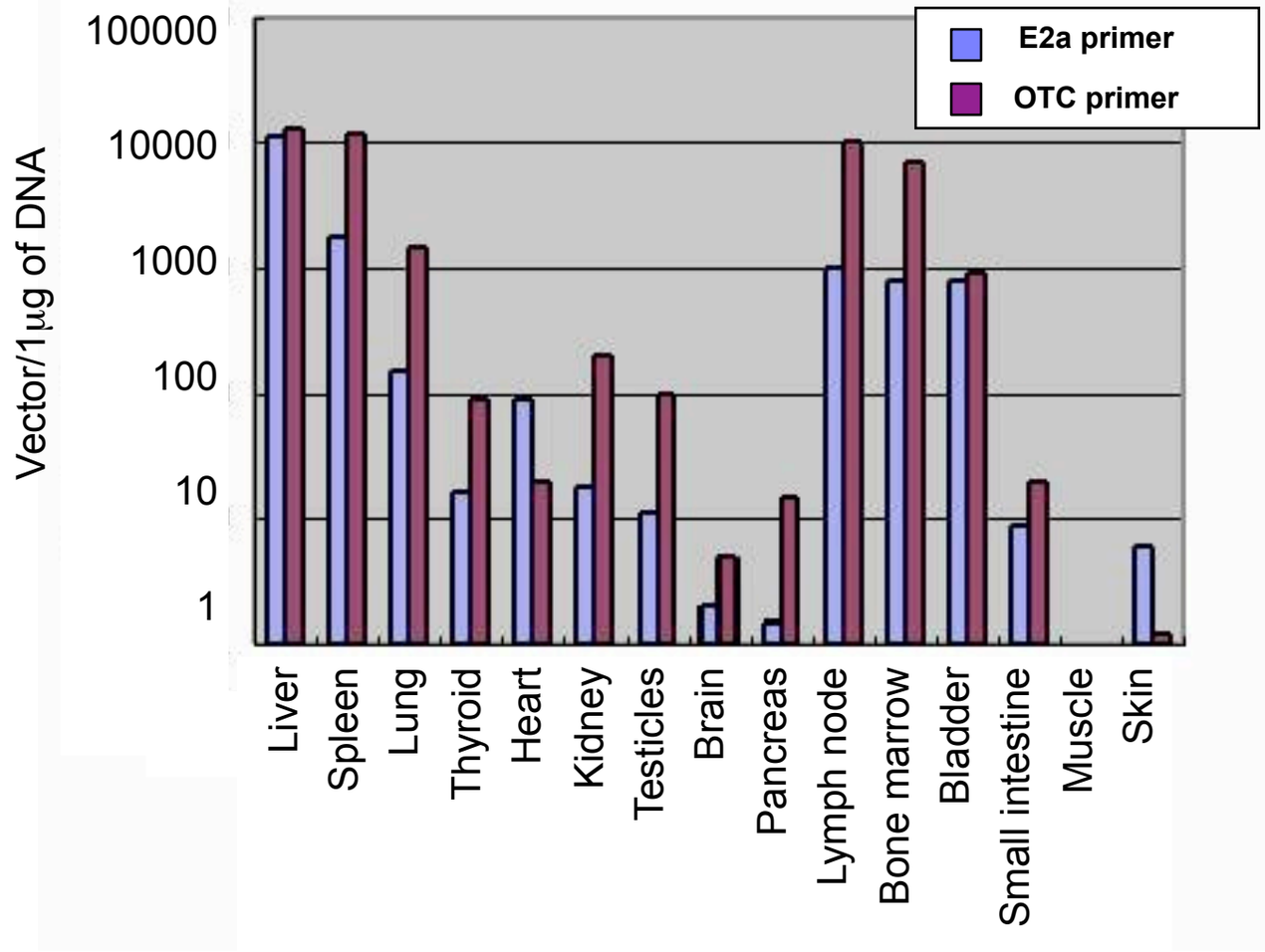
Target diseases for gene therapy

Cancer, Enzyme-deficiency, AIDS, Cardiovascular diseases, Diabetes, Tissue regeneration, etc.



Gene Therapy Death Prompts Review of Adenovirus Vector





Traces of adenovirus DNA (E2a) and a curative gene (OTC). Patient's target organ is the liver

- **Inherent antigenicity**
- **Limited size in encapsulated gene**
- **Difficulties in large-size production**

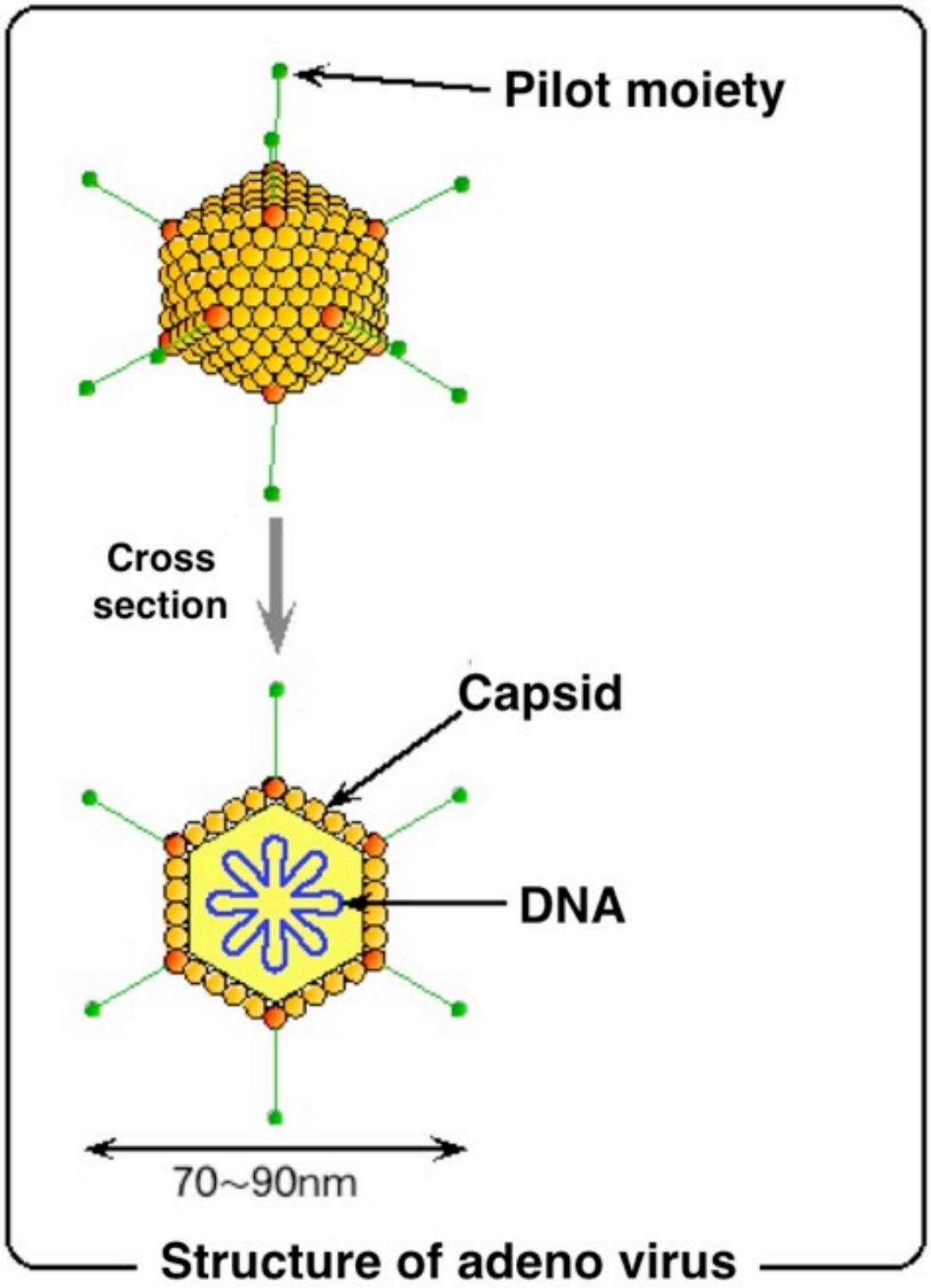
Gene therapy death of patients at University of Pennsylvania on September, 1999

→38 trillion virus particles were dosed through i.v. route, yet only 1% of the transferred genes reached the target cells

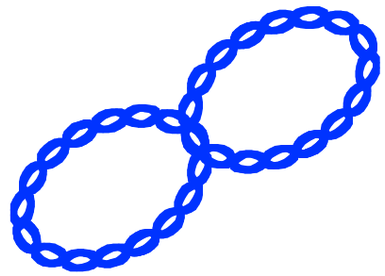
→Restricted clinical use of adenovirus vector

Science 286, 2244(1999)

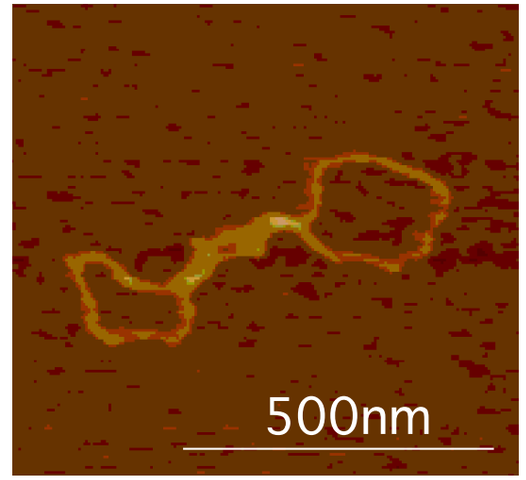
pDNA entrapped polymeric micelle for gene delivery

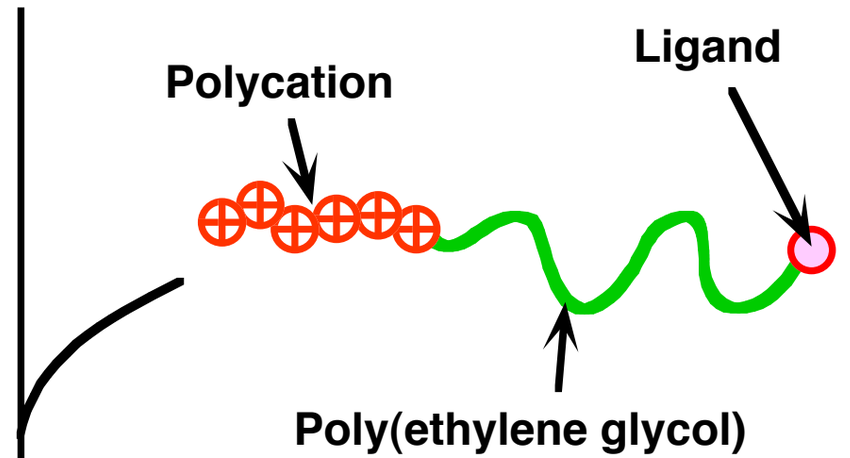


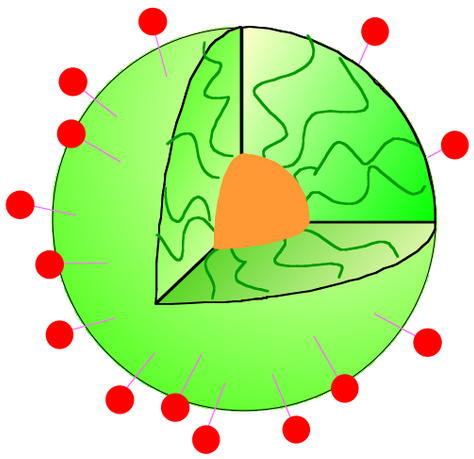
Structure of adeno virus

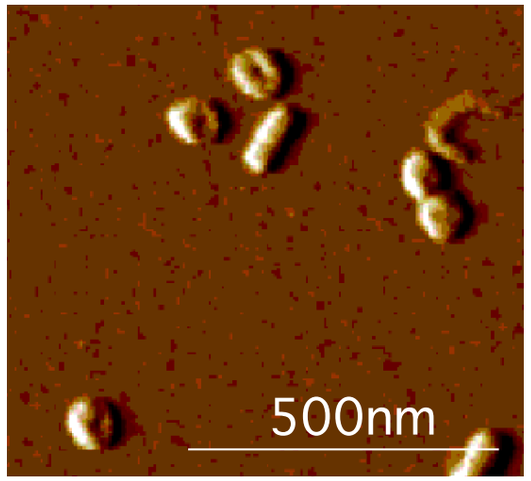


Plasmid DNA



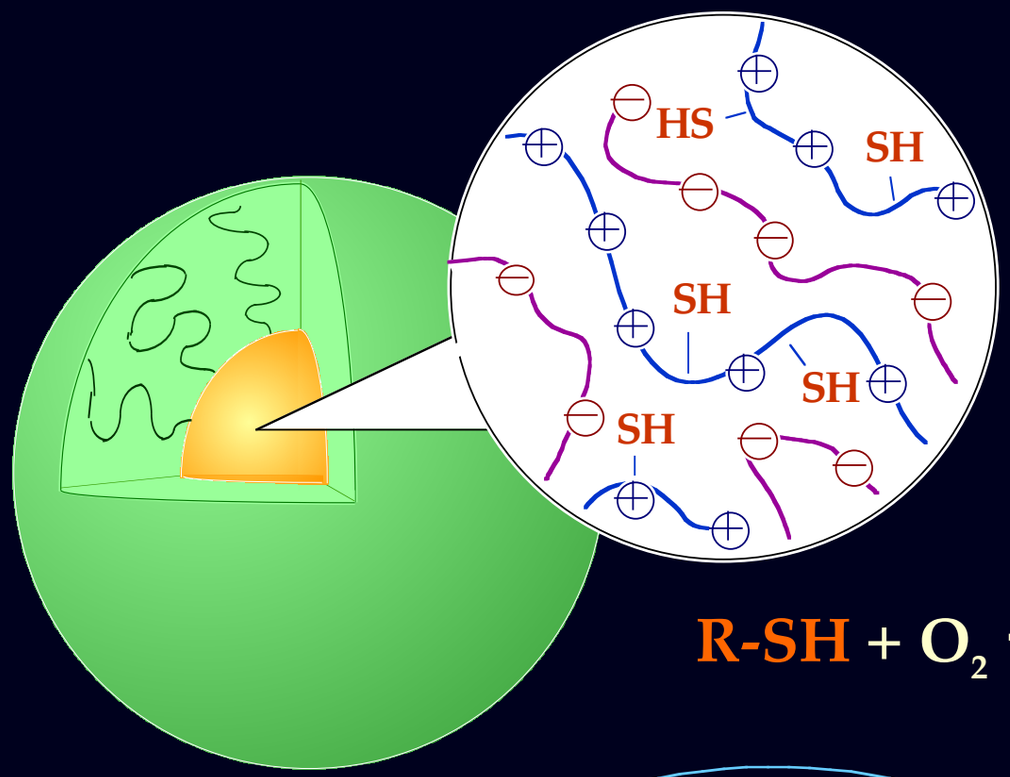






S. Katayose, et al, *Bioconjugate Chemistry*, 8, 702 (1997); K. Itaka, et al, *Biomaterials*, 24, 4495 (2003)

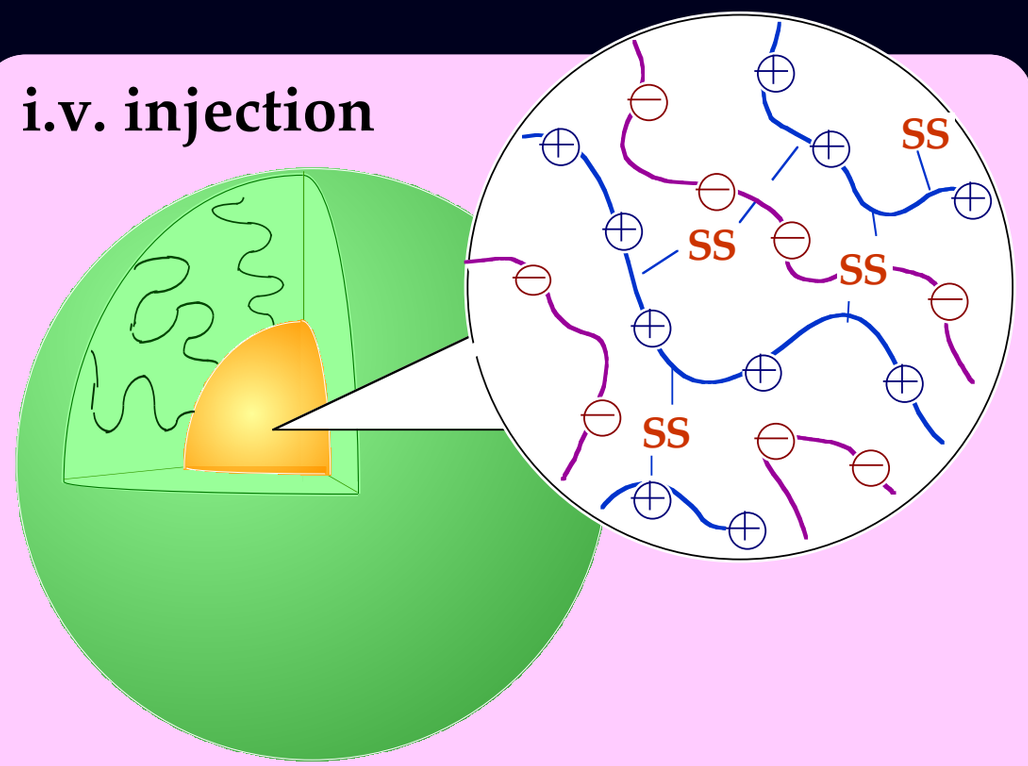
Environment-sensitive stabilization of core-shell structured PIC micelle by reversible cross-linking of the core through disulfide bond



O_2

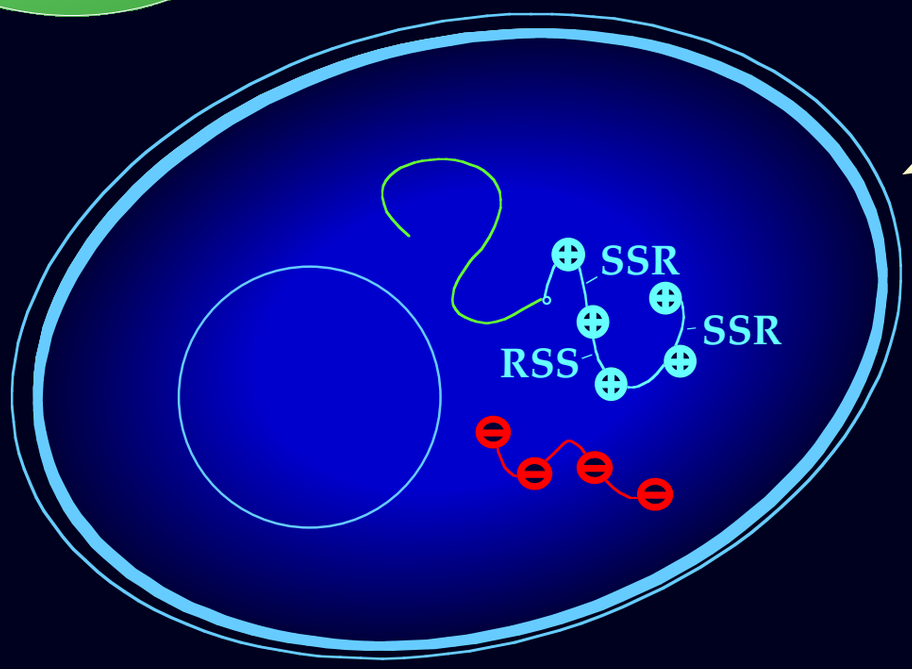
$R\text{-}SH + O_2 \rightarrow R\text{-}SS\text{-}R$

i.v. injection



Stabilization in the blood stream
[glutathione] = 10 μM

R'-SH



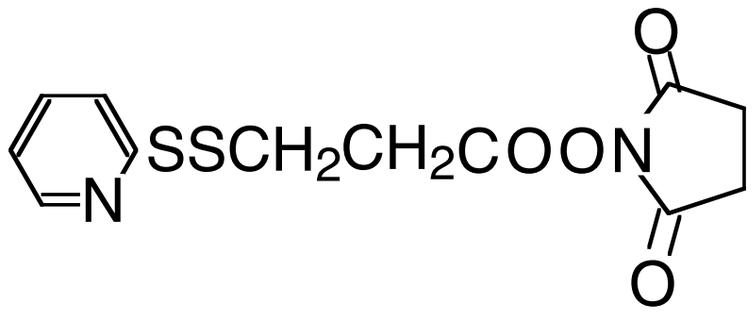
Dissociation inside the cell
[glutathione] = 3 - 5 mM

$R\text{-}SS\text{-}R + R'\text{-}SH \rightarrow R\text{-}SH + R\text{-}SS\text{-}R'$

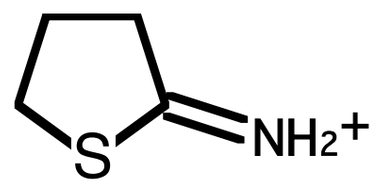
Y. Kakizawa, et al, JACS, 121, 11247 (1999); K. Miyata, et al, JACS, 126, 2355 (2004); K. Miyata, et al, J. Contrl. Rel., 109, 15 (2005)

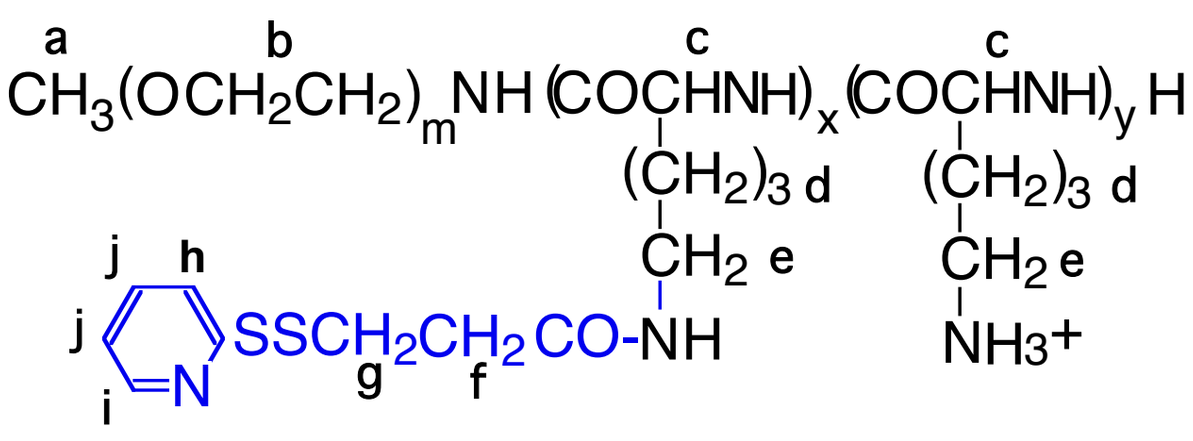
Preparation of thiolated PEG-PLL with controlled charge density

SPDP

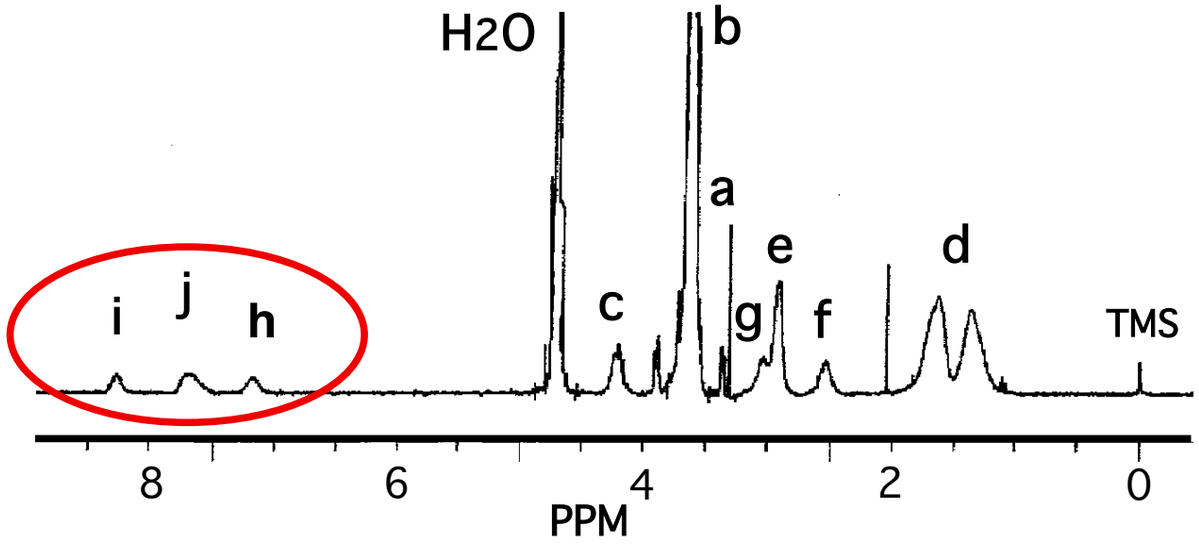


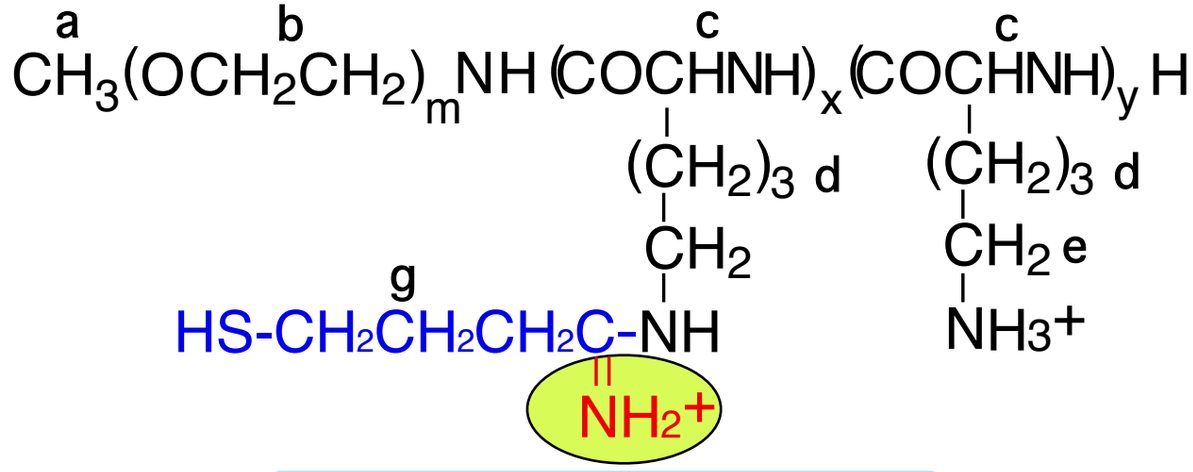
2-iminothiolane



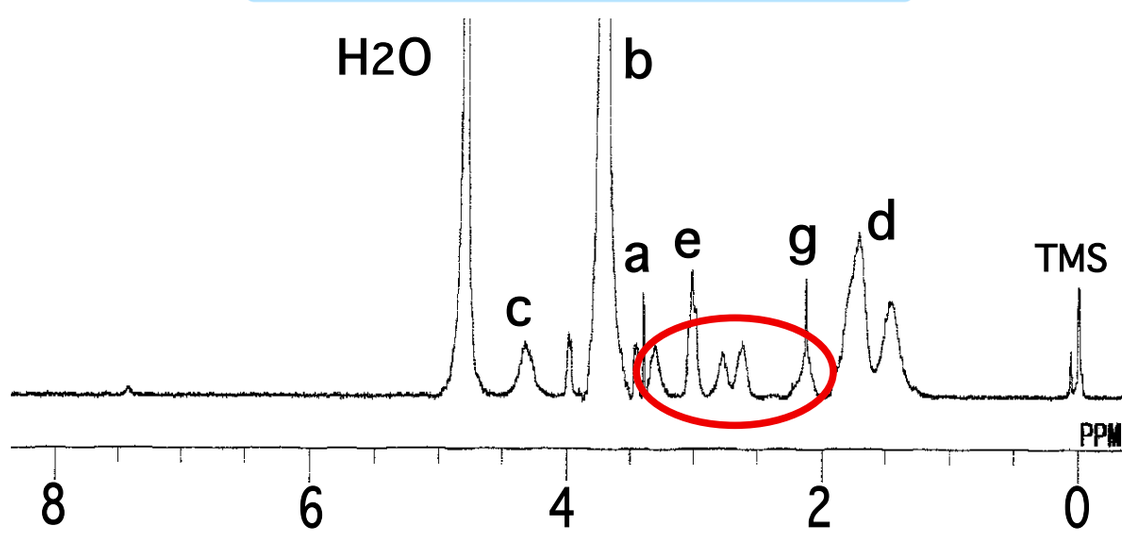


SP−X(X: SS mol%)

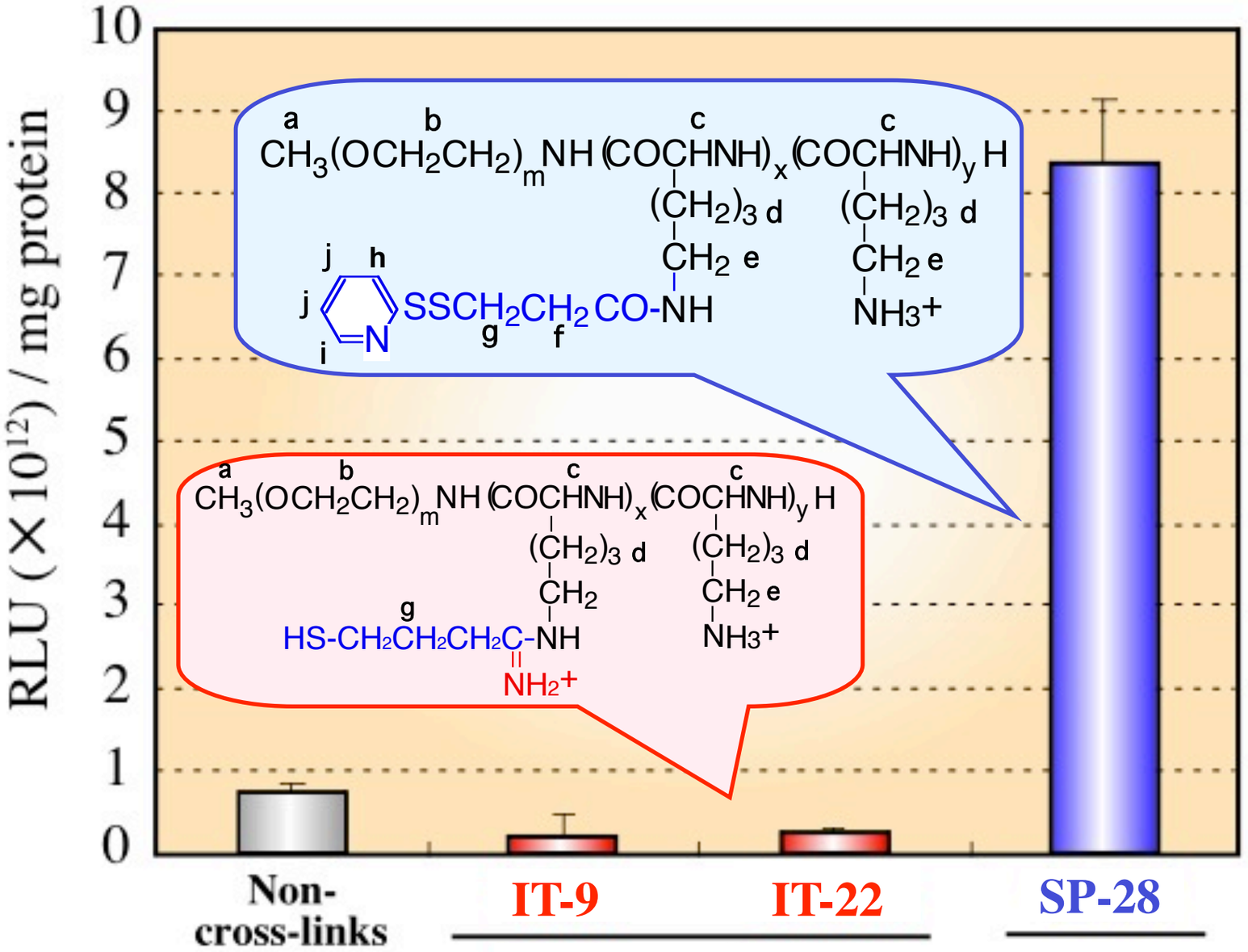




IT−X(X: SH mol%)



Improved Gene Transfection by SS Crosslinked Micelles



293T cells

Improved stimuli sensitivity through charge compensated introduction of SS crosslinking

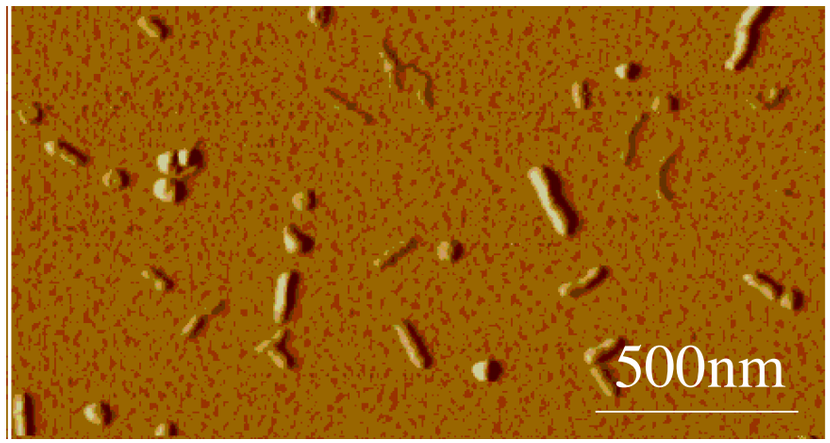
Remarkable increase in transfection efficiency

K. Miyata, et al, *J. Amer. Chem. Soc*, 126, 2355-2361 (2004)

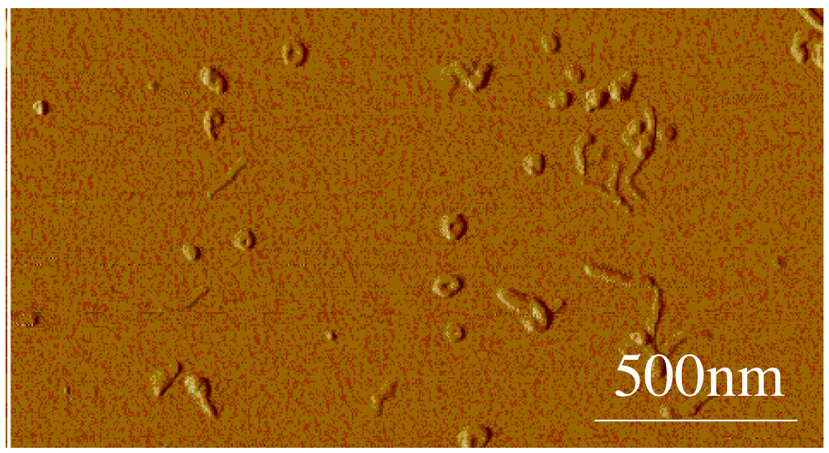
Enhanced gene transfection by freeze-dryable cross-linked micelle vector

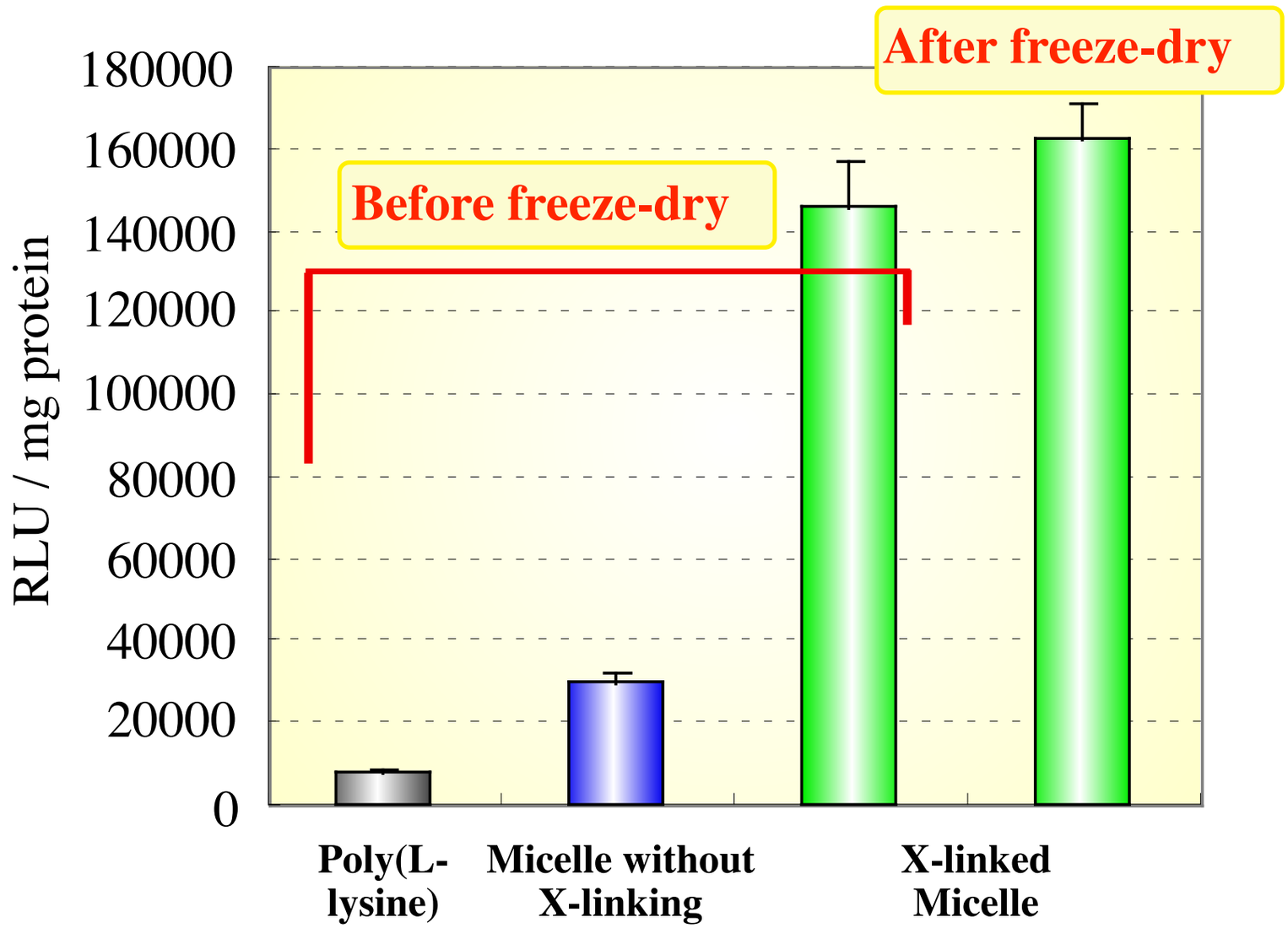
	Poly(L-lysine)	Micelle without X-linking	X-linked Micelle
Size before freeze-dry (nm)	105.9	98.1	114.3
Size after freeze-dry (nm)	(1744.8)	(2176.5)	127.2

AFM of X-linked micelle



↓ After freeze dry

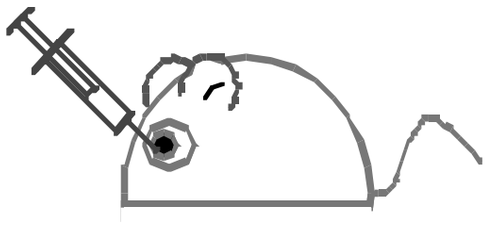




K. Miyata, et al, *J. Amer. Chem. Soc*, 126, 2355-2361 (2004); K. Miyata, et al, *J. Contrl. Rel.*, 109, 15-23 (2005)

Liver transfection by systemic injection of cross-linked micellar vector

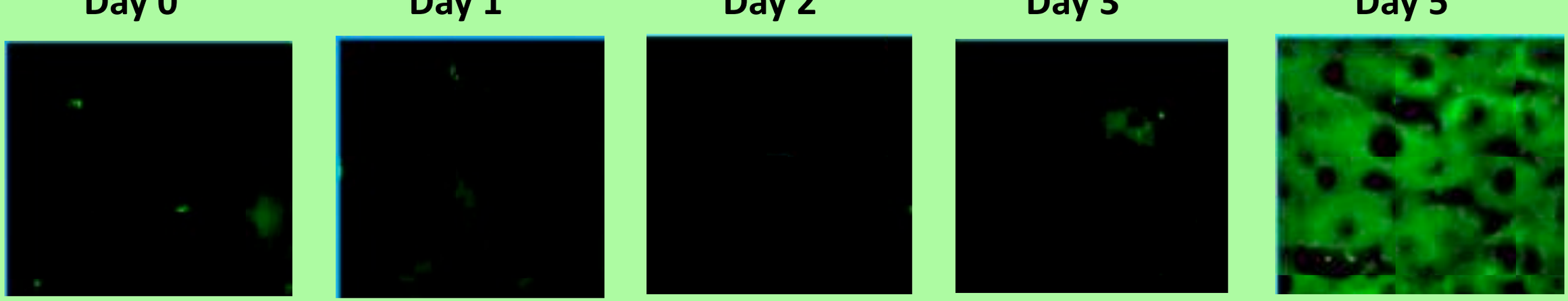
i.v. injection via orbital vein



→ **Liver was excised at a defined day** → **The excised liver was treated wit OCT compound, and sliced with cryostat.**

YFP gene expression in liver

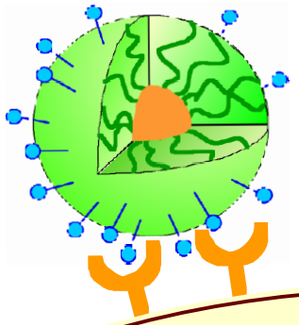
Day 0 **Day 1** **Day 2** **Day 3** **Day 5**



Homogeneous YFP gene expression was observed in liver parenchymal cells 5 days after i.v. injection of cross-linked polyplex micelles.

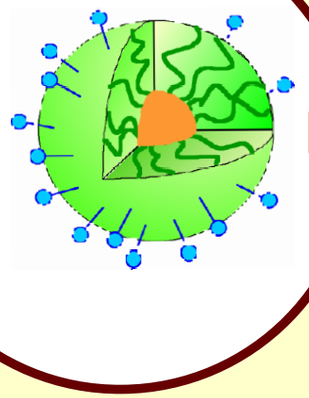
K. Miyata, et al, *J. Contrl. Rel.*, 109, 15-23 (2005)

Endosomal escape: A key issue in intracellular gene and nucleic acid delivery



Gene vector

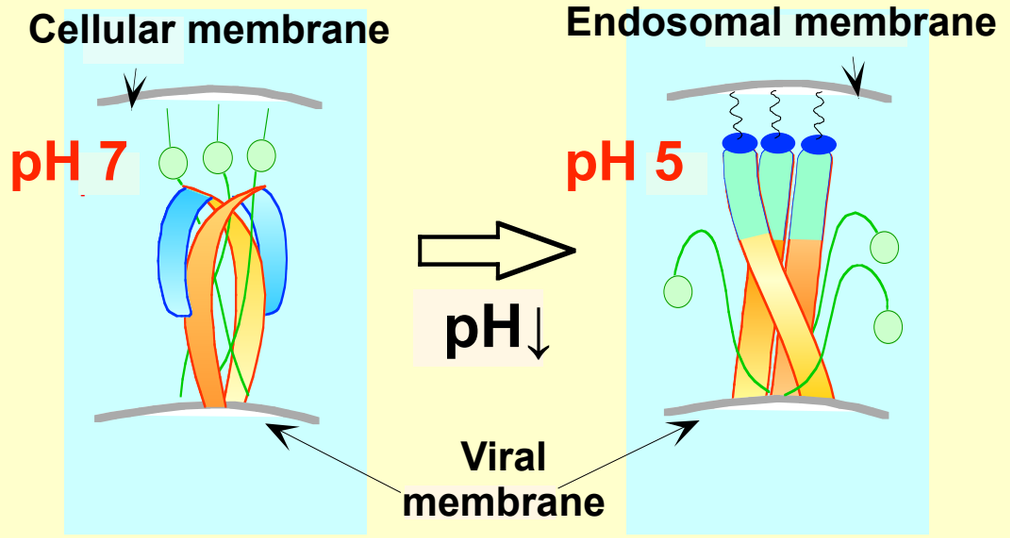
Endocytosis



Endosome escape

Gene expression

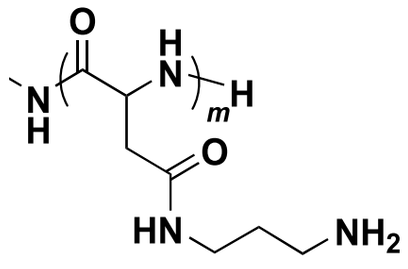
Cell

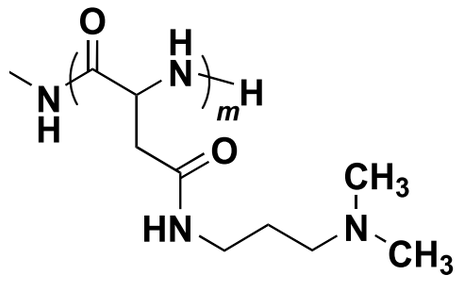


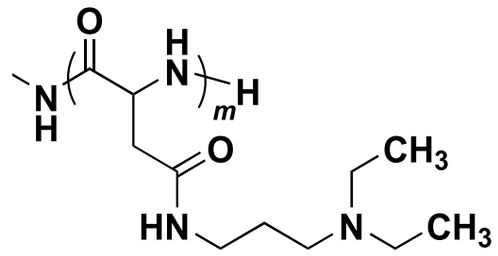
Membrane fusion by pH-sensitive peptide domain (Ex. Influenza virus)

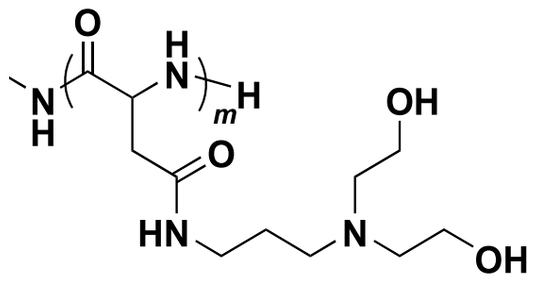
Challenge: Integrating endosome escaping units with minimum cytotoxicity into polyplex nanocarrier

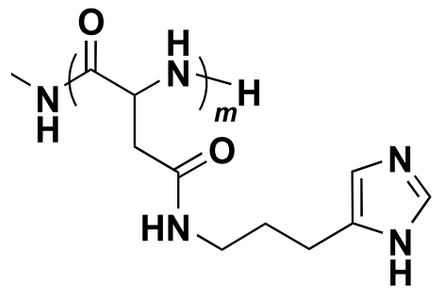
Preparation of a Series of Cationic Polyaspartamides through Aminolysis Reaction of Poly(beta-benzyl aspartate)

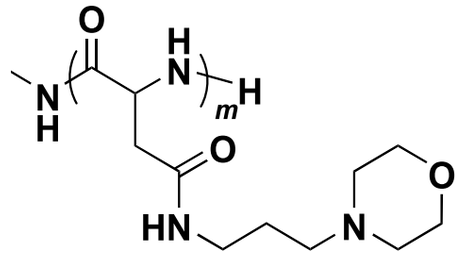


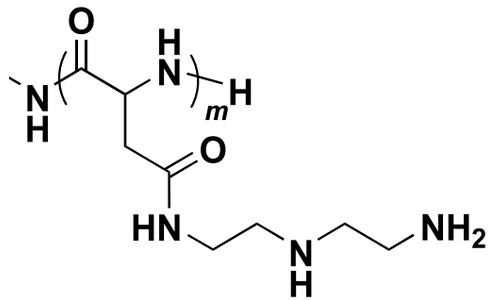




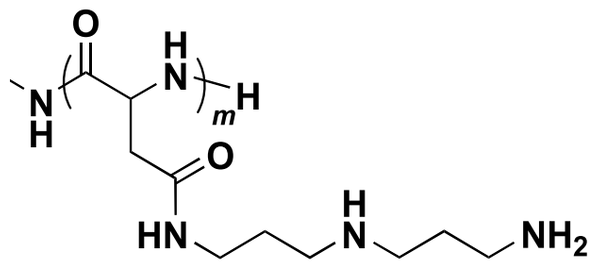


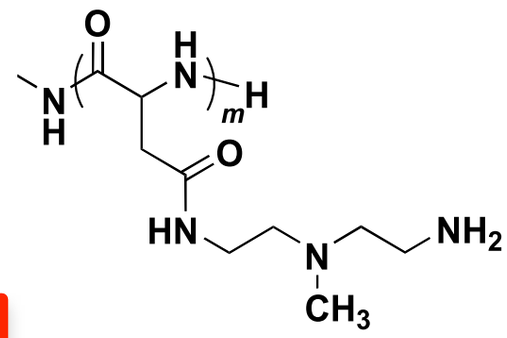


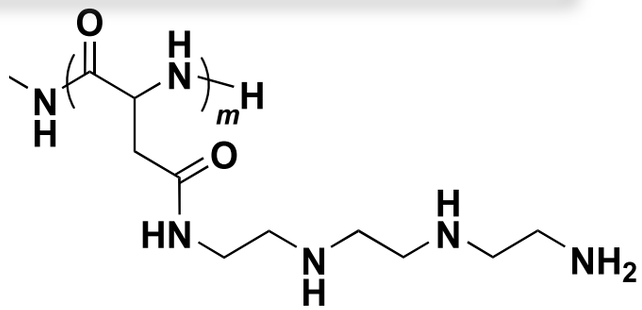


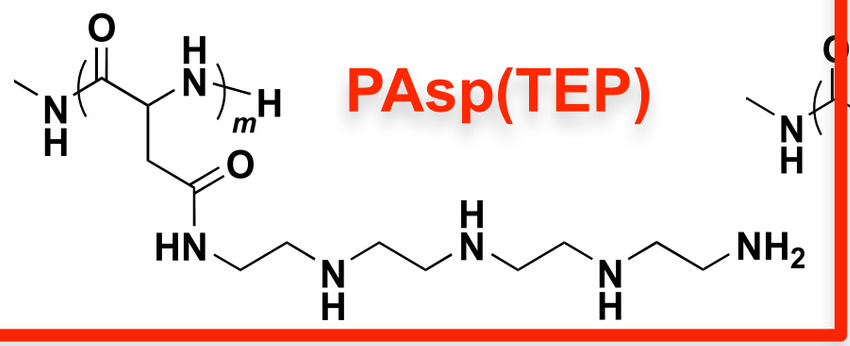


PAsp(DET)

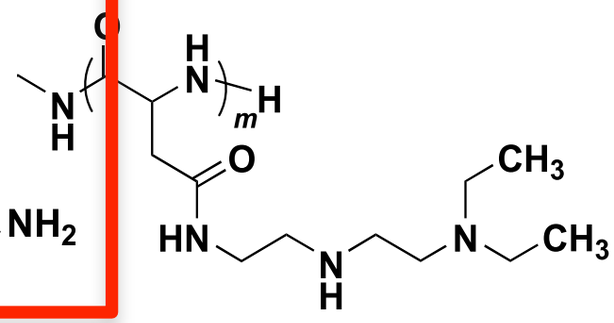








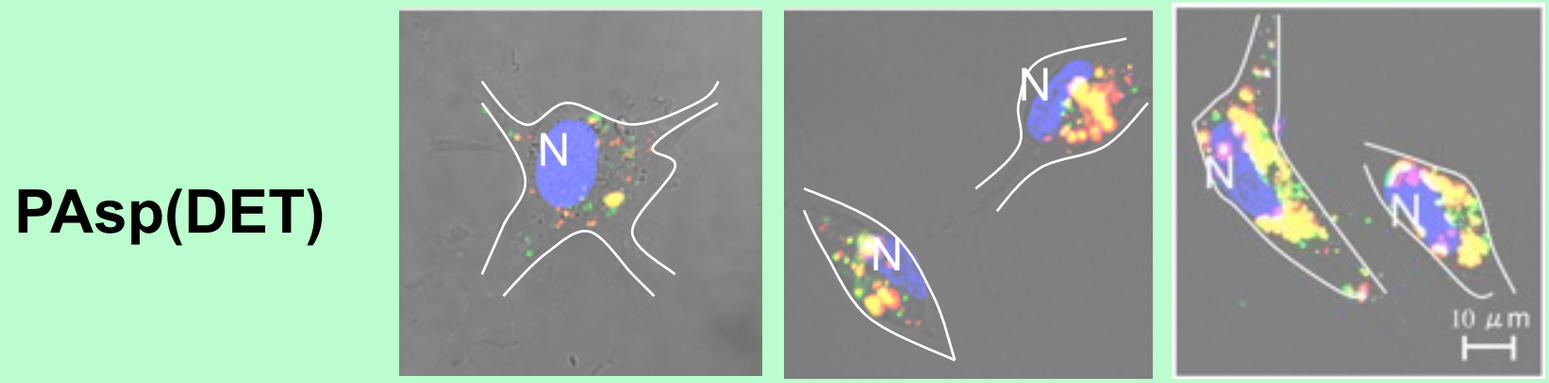
PAsp(TEP)

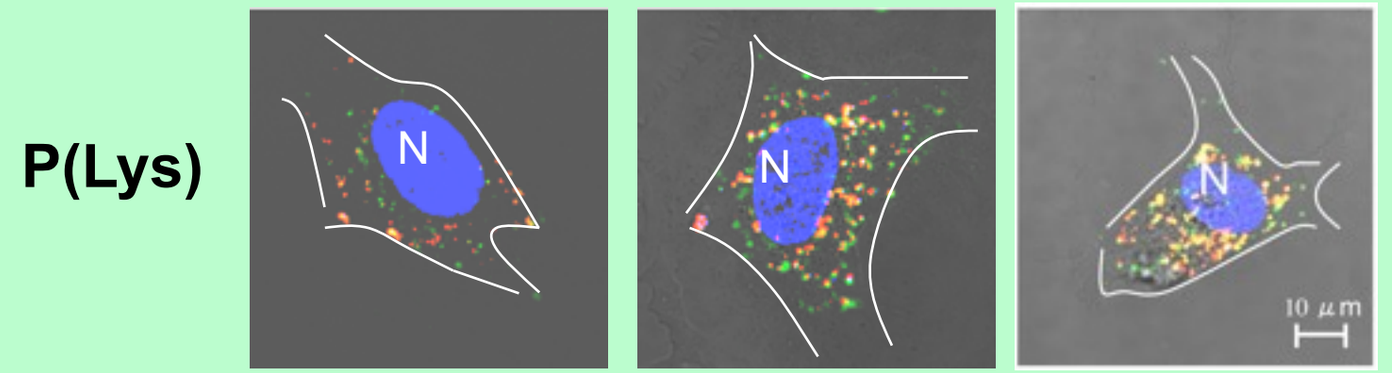


ChemMedChem 1, 439-444 (2006); *J. Cotrl. Rel.* **115** *208-215 (2006); React. Funct. Polym*. 67, 1361-1372 (2007)

Preparation of a Series of Cationic Polyaspartamides through Aminolysis Reaction of Poly(beta-benzyl aspartate)

Observation of endosomal escape



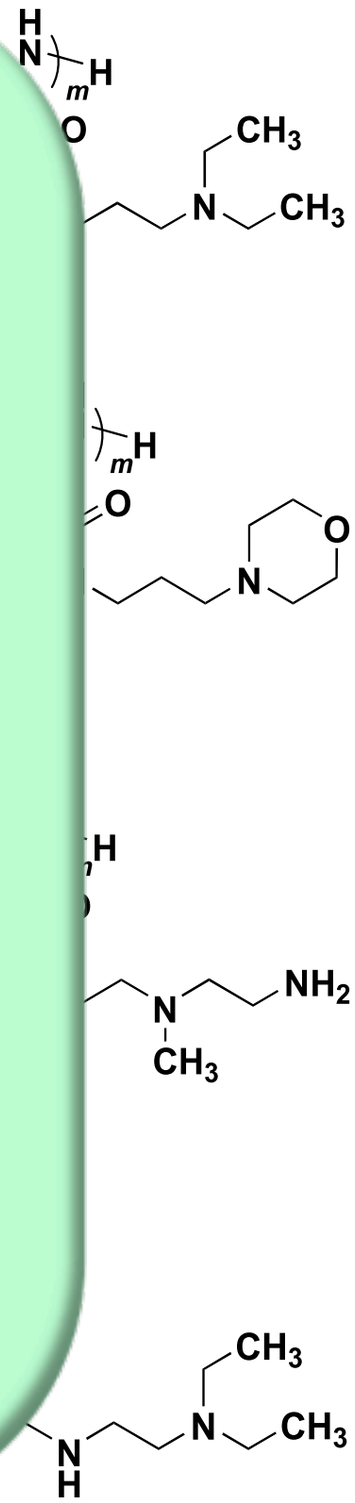


Green: Alexa-labeled dextran (endosome marker)

Red: Cy5-labeled pDNA

Blue: Hoechst 33258-stained nucleus

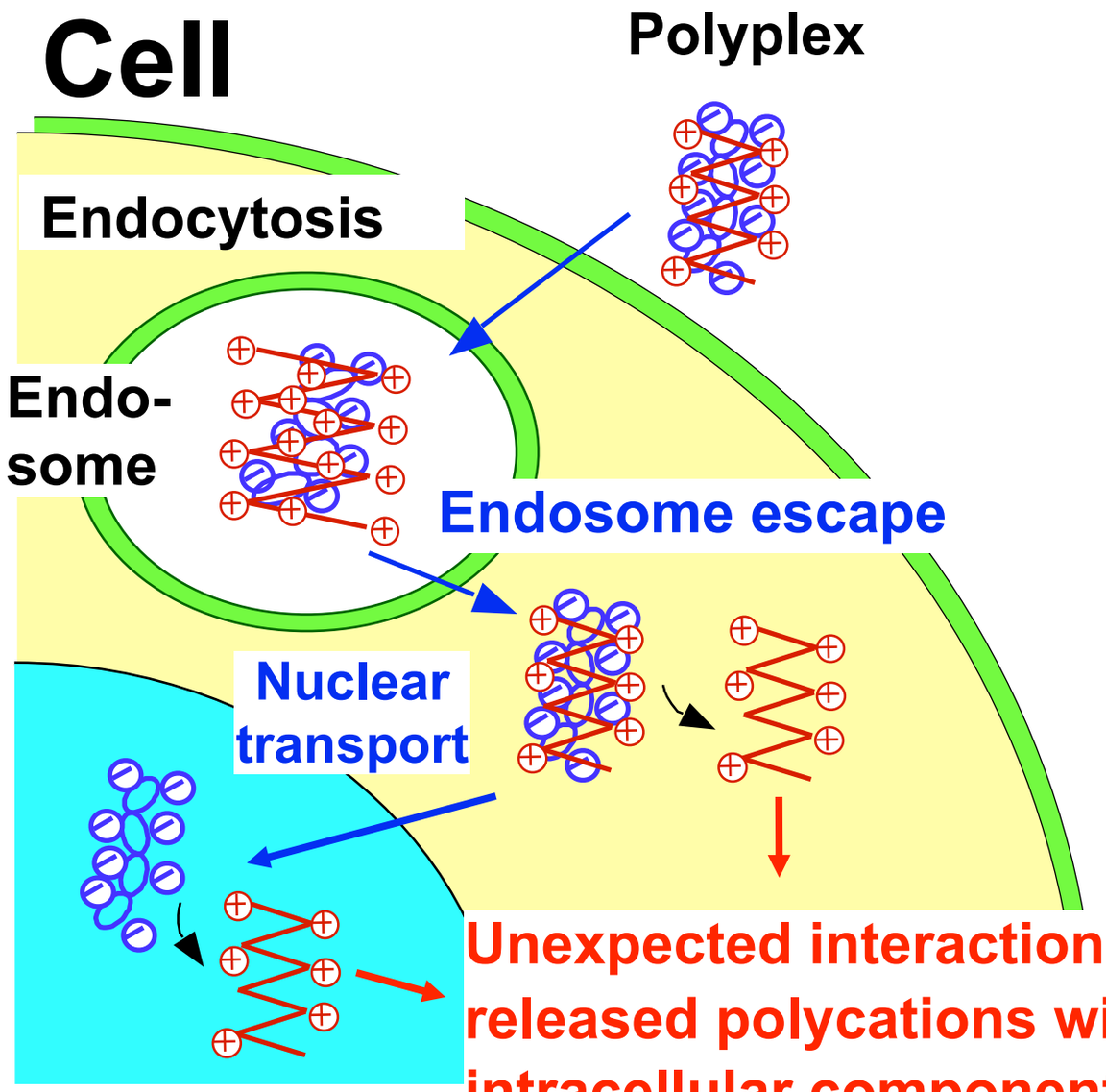
K. Miyata, et al, *J. Amer. Chem. Soc.*, 130, 16287-16294 (2008)



ChemMedChem 1, 439-444 (2006); *J. Cotrl. Rel.* **115** *208-215 (2006); React. Funct. Polym*. 67, 1361-1372 (2007)

Biocompatibility assay of polycations by materials genomics

Adverse effect of polycations in polyplex due to the non-specific interaction with intra-cellular components

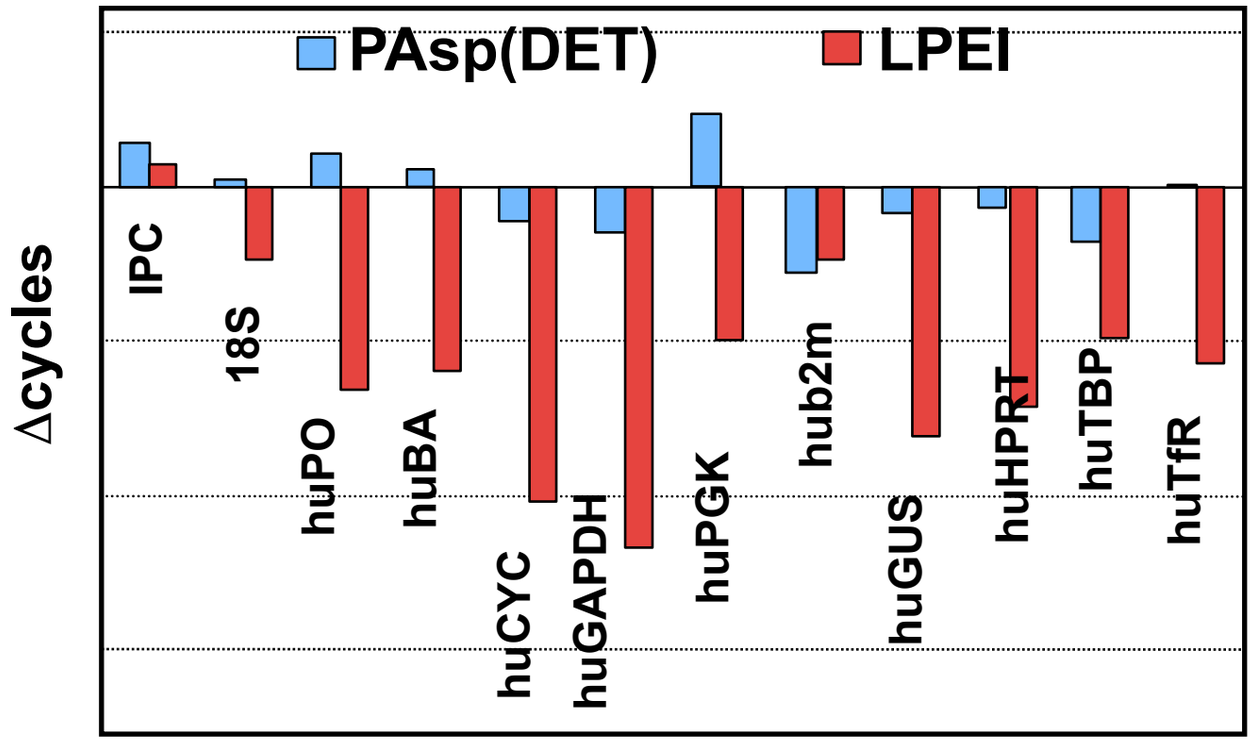


Unexpected interaction of released polycations with intracellular components

Impaired homeostasis

K. Masago, et al, *Biomaterials*, 28, 5169 (2007)

Change in the expression of house-keeping genes with polyplex transfection (Evaluation by real-time PCR)

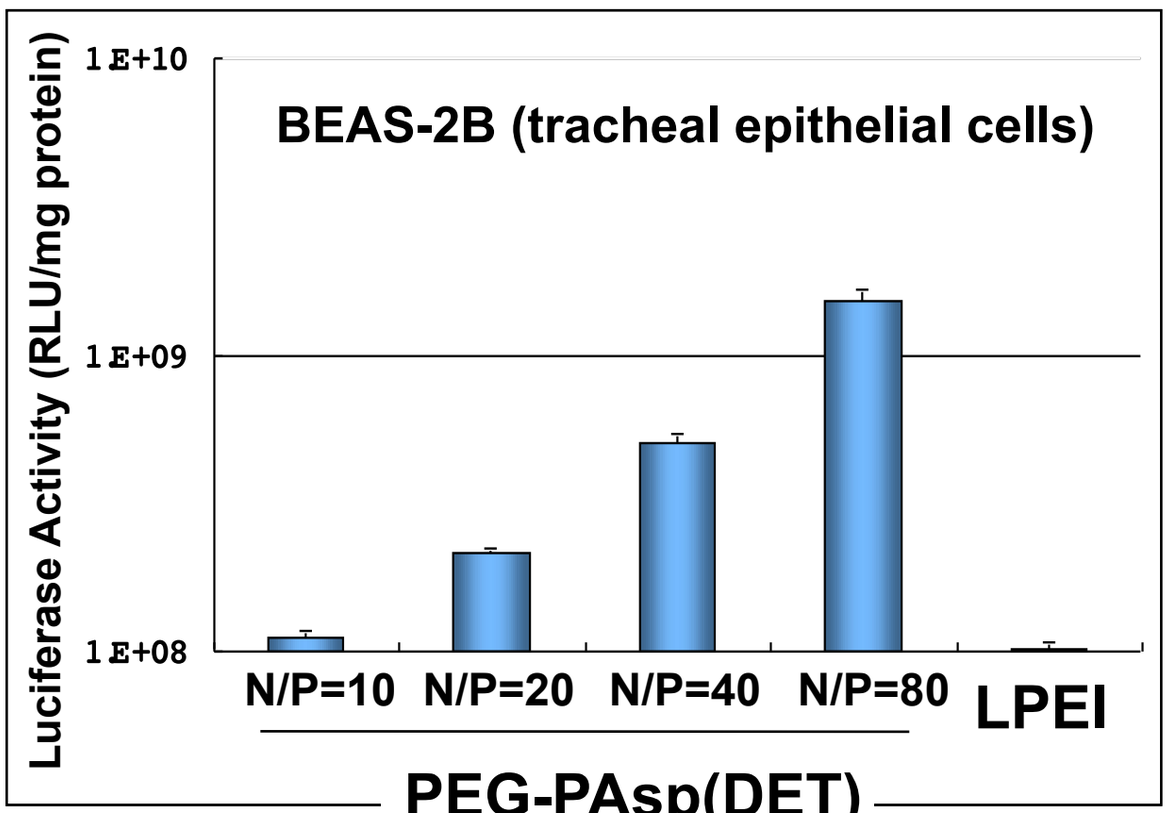


Significant decrease in the house-keeping gene expression for LPEI, yet no significant change for PAsp(DET)

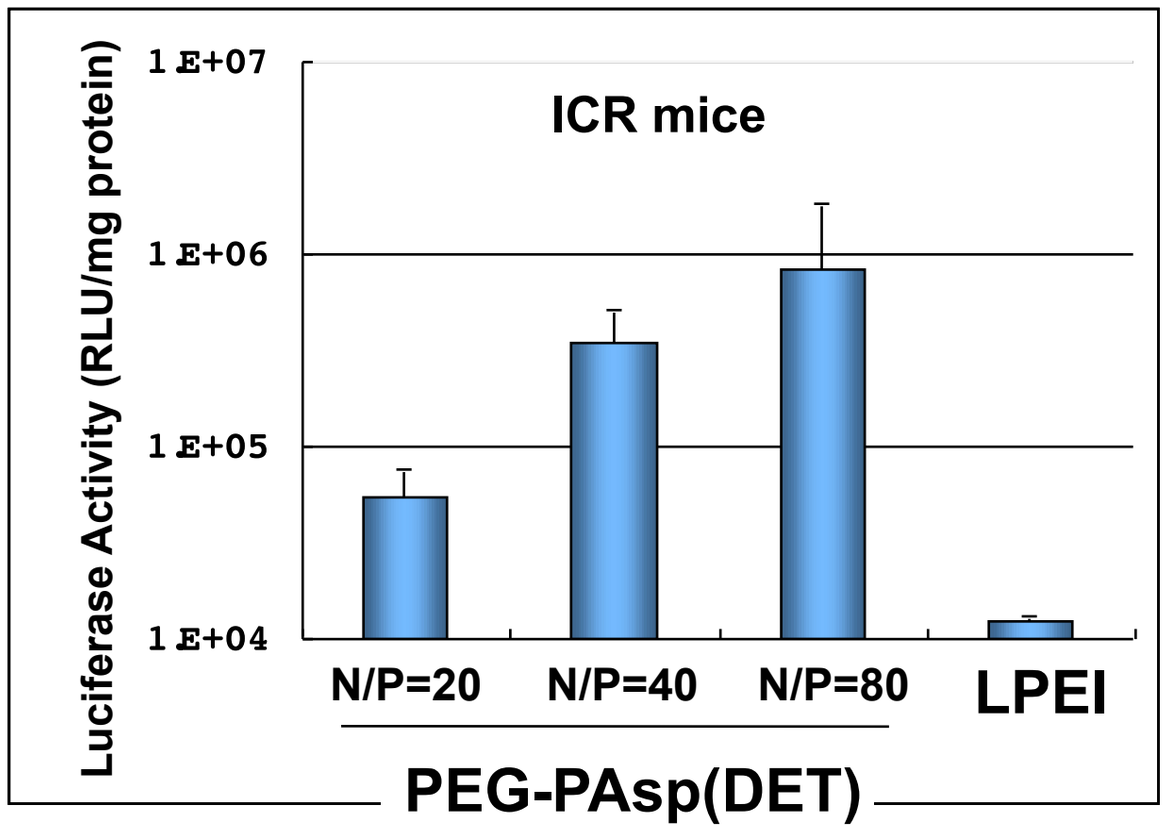
PAsp(DET) as biocompatible gene carrier from the standpoint of materials genomics

Gene Transfer to Lung by PEG-PAsp(DET) Polyplex Micells

Luciferase gene transfection to tracheal epithelial cells (in vitro)



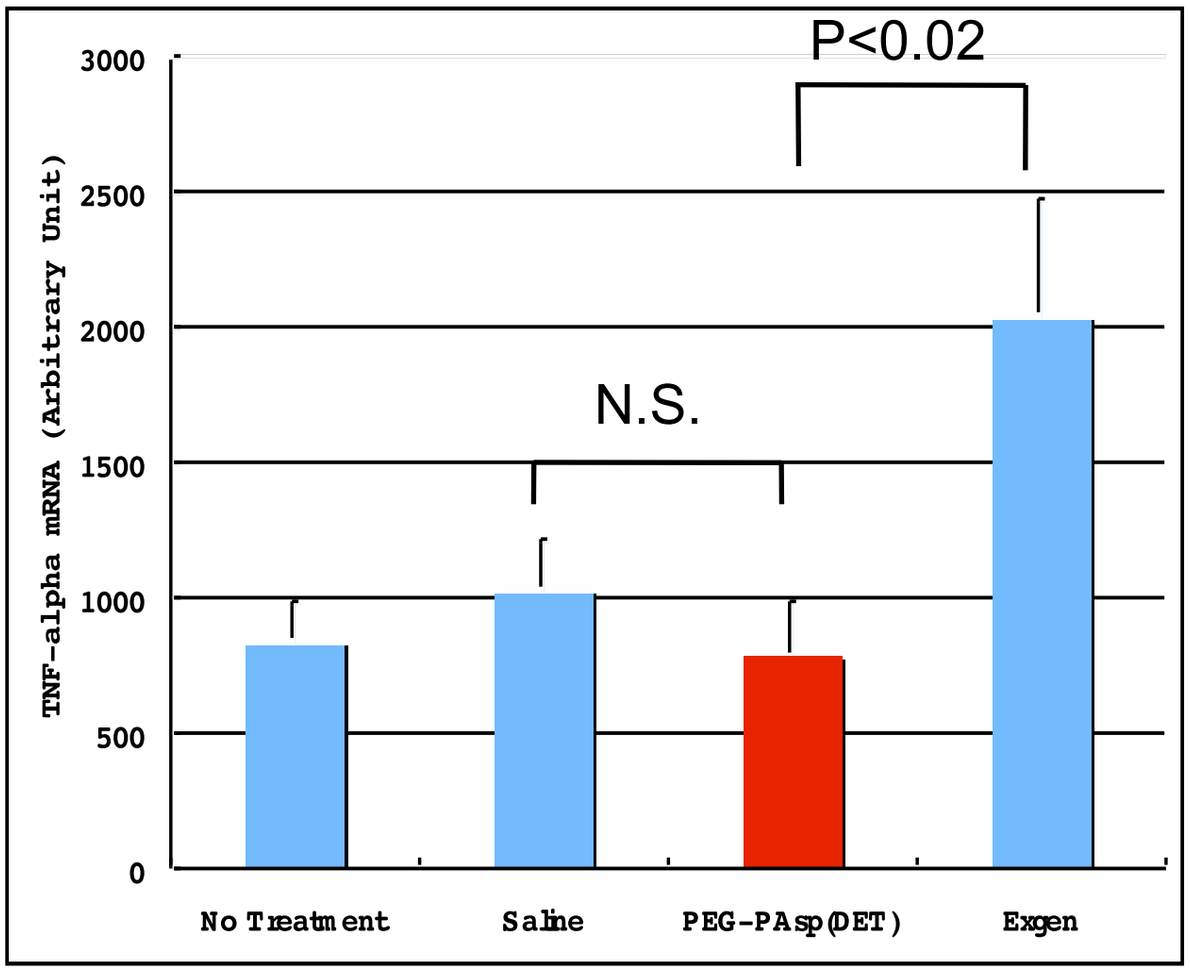
Luciferase gene transfection to lung via intratracheal administration (in vivo)



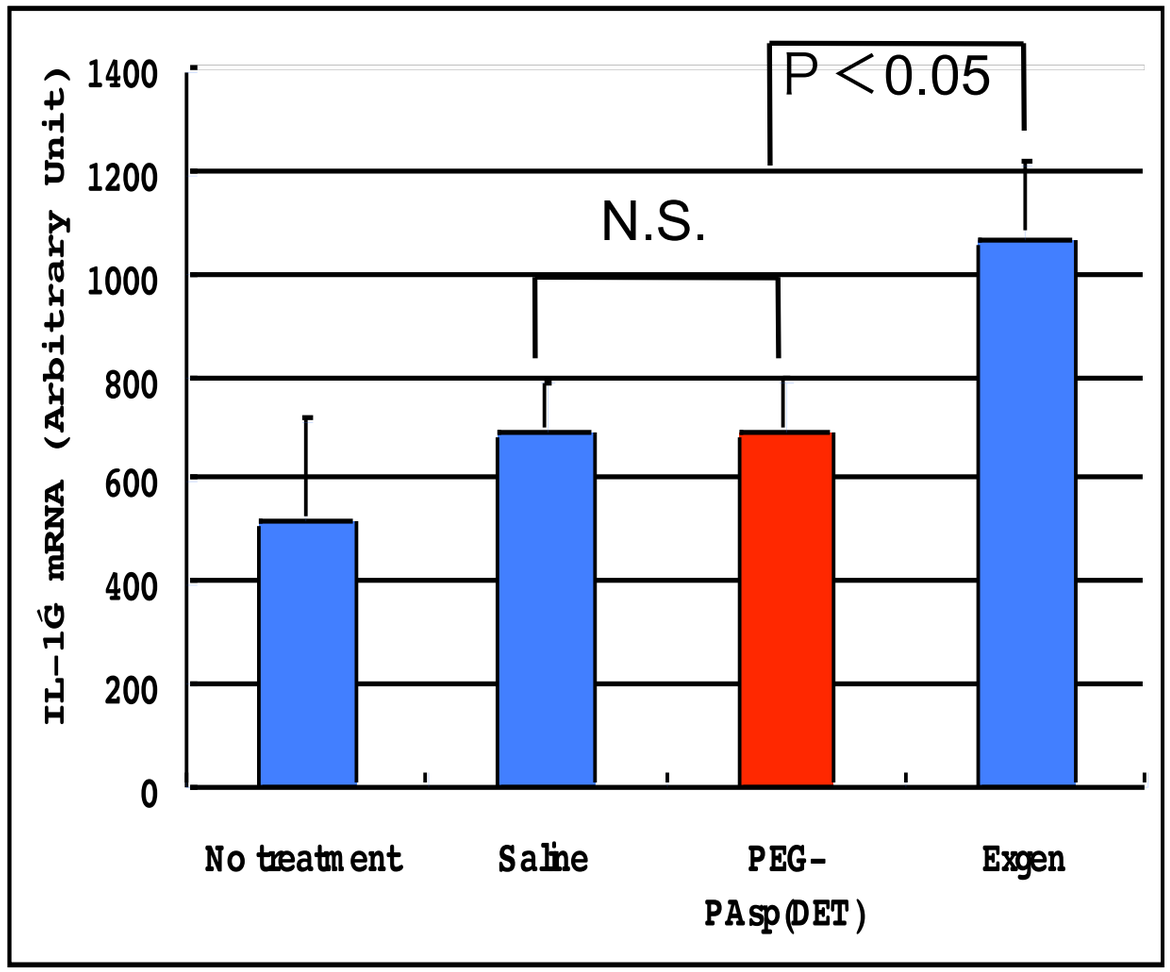
M. Shiba, et al, *Molecular Therapy,* 17(7), 1180-1186 (2009)

mRNA expression of inflammatory cytokines (TNF-α and IL-1β) in the lung 1 week after the administration of polyplexes

TNF-α



IL-1β



M. Shiba, et al, *Molecular Therapy,* 17(7) 1180-1186 (2009)

Adrenomedullin Gene Transfer by Intratracheal Administration of PEG-PAsp(DET) for the Treatment of PAH

Model Animals for Pulmonary Hypertension

Day 0
- Subcutaneous injection of monocrotaline

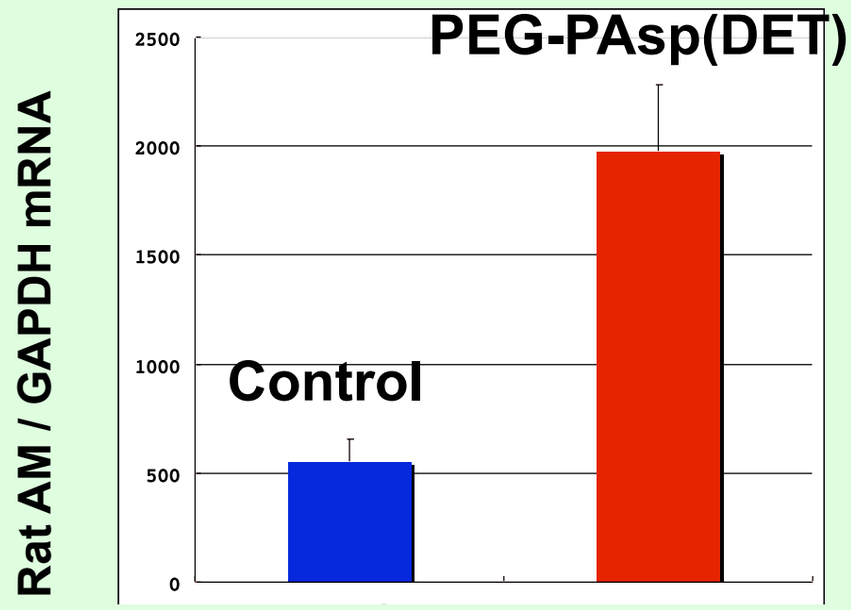
Day 25
- Measurement of right ventricular pressure by catheter
- Gene transfer of AM gene by intratracheal administration

Day 28
- Measurement of right ventricular pressure by catheter again and remove of the lung to measure RNA

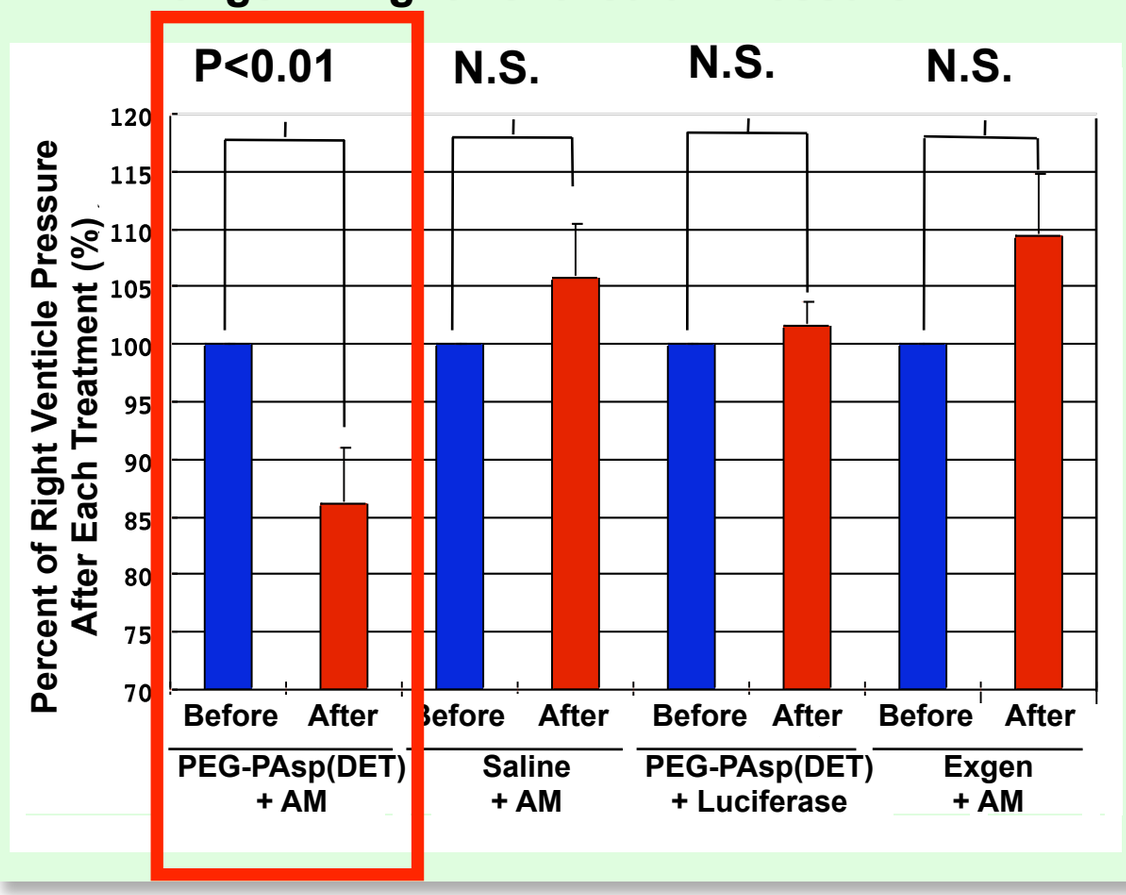
Pulmonary Arterial Hypertension

- Life-threatening disease characterized by progressive pulmonary arterial hypertension
- Death after 2 to 10 years of diagnosis.

Measurement of adrenomedullin mRNA by Real Time RT-PCR



Change in Right Ventricular Pressure



M. Shiba, et al, *Molecular Therapy*, 17(7) 1180-1186 (2009)

Trinity in Regenerative Medicine

Cell

- ES cell
- MSC cell
- iPS cell

Differentiation Control

- Drug
- Gene, siRNA
- Bioactive substance

Scaffold

- PLLA, PLGA
- Calcium Phosphate
- Collagen

Need for drug and gene delivery systems

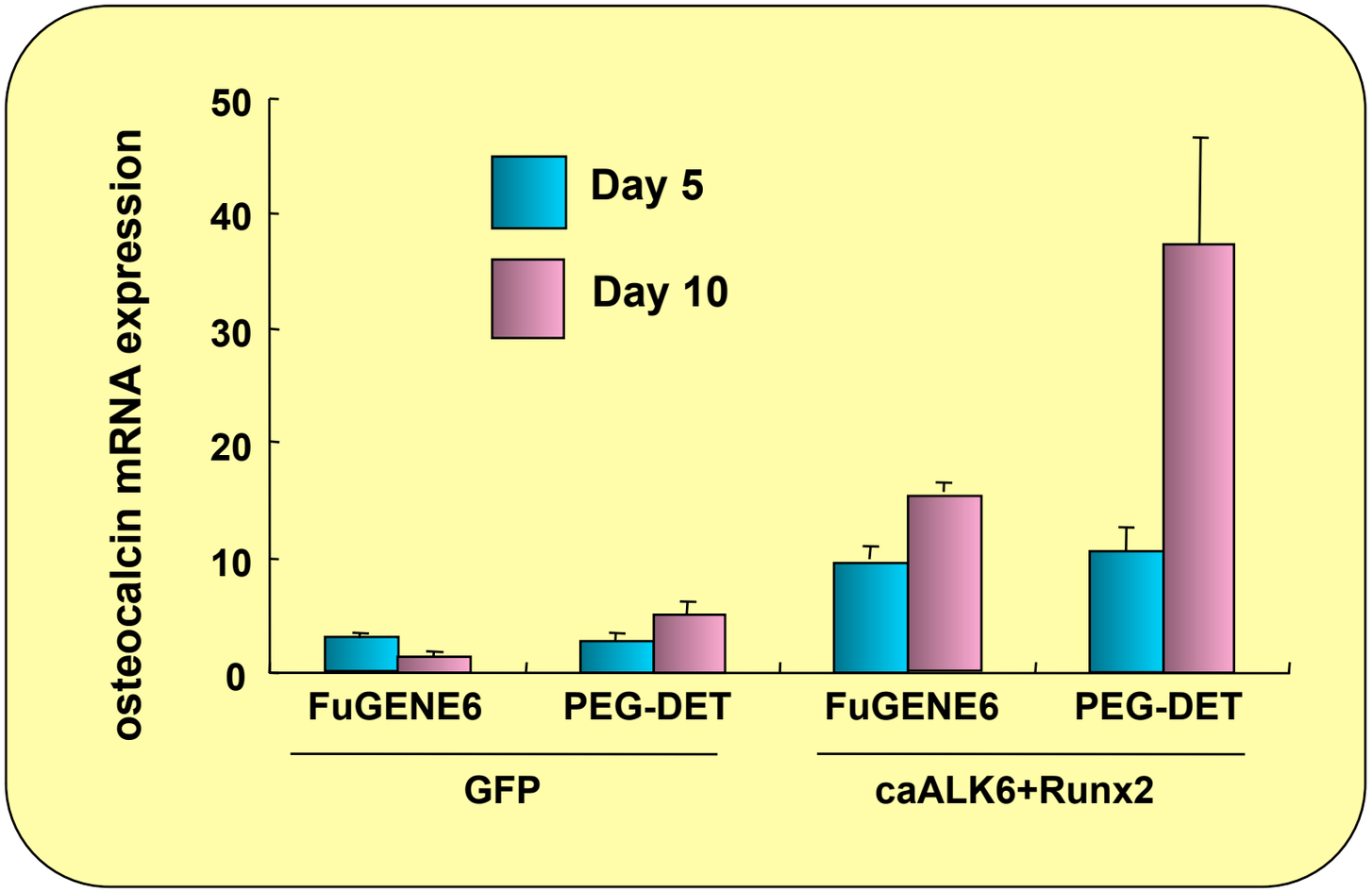
Induction of cell differentiation by delivering genes encoding osteogenic factor with polyplex micelles

pDNA encoding osteogenic factor (caALK6+Runx2)

Primary osteoblast

5, 10days

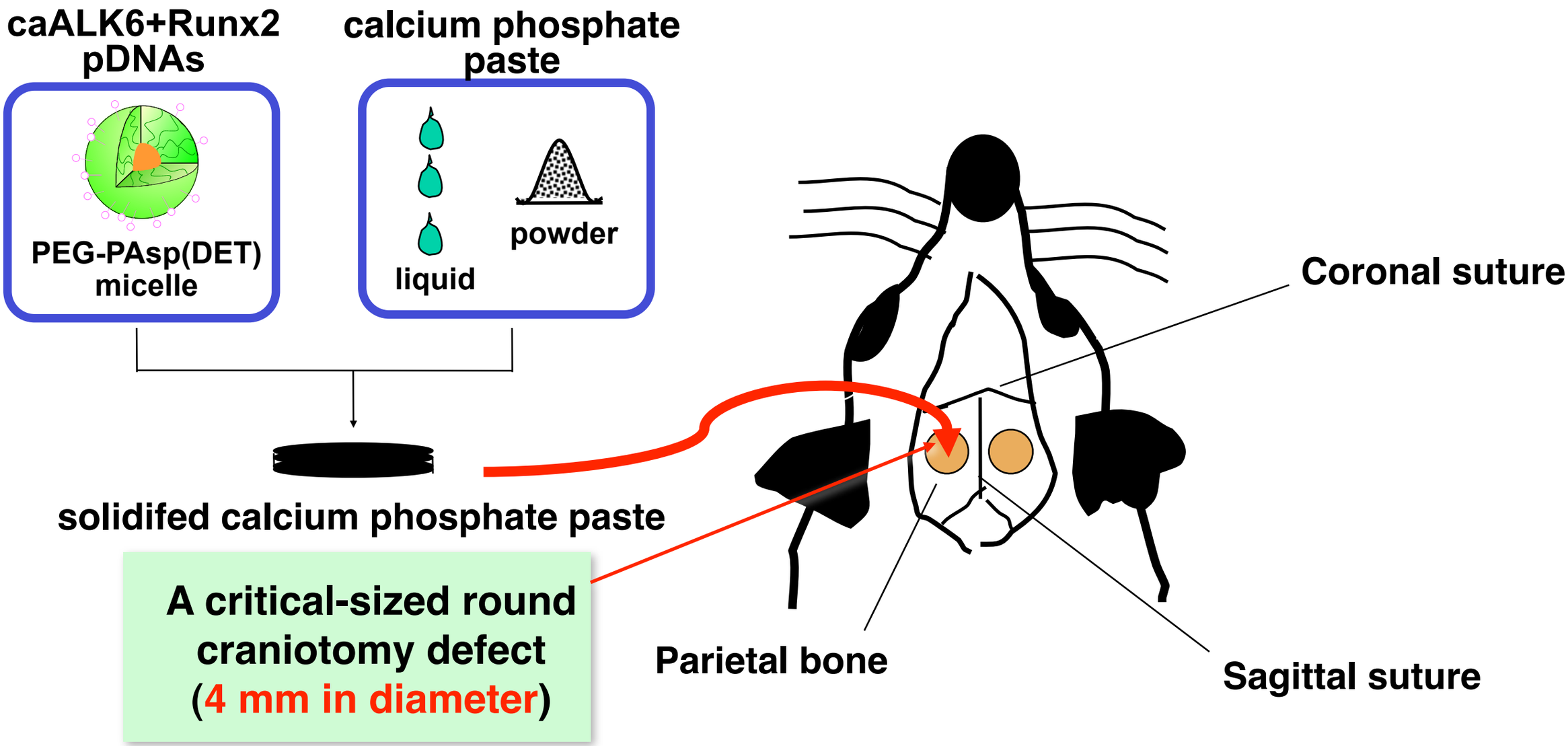
Estimation of osteocalcin (osteoblast differentiation marker) mRNA expression by real-time RT-PCR



Only polyplex micelles successfully induce the cell differentiation.

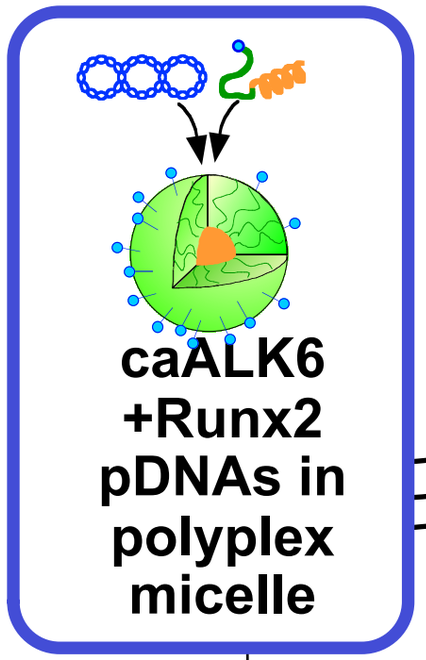
K. Itaka, et al,
Molecular Therapy, 15, 1655-1662 (2007)

Bone regeneration based on *in vivo* transduction without cell source



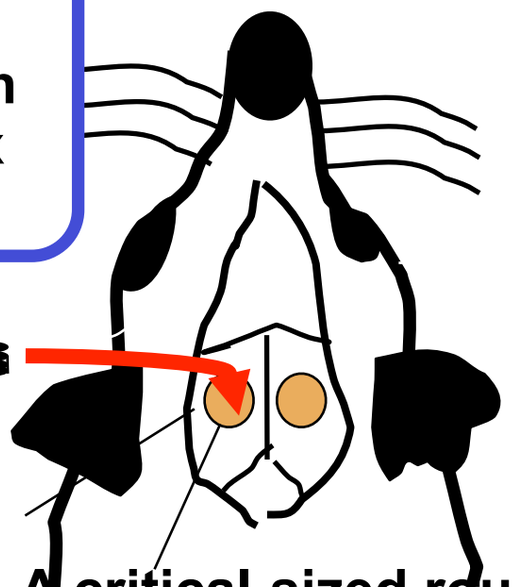
- **Solidified calcium phosphate paste containing Runx2 and caALK6-expressing pDNAs (1.3 μg/mouse) were placed to cover the defects.**
- **The mice were sacrificed at 2, 4, 6 weeks after the operation for histological analyses.**

Bone regeneration based on *in vivo* transduction without cell source



caALK6
+Runx2
pDNAs in
polyplex
micelle

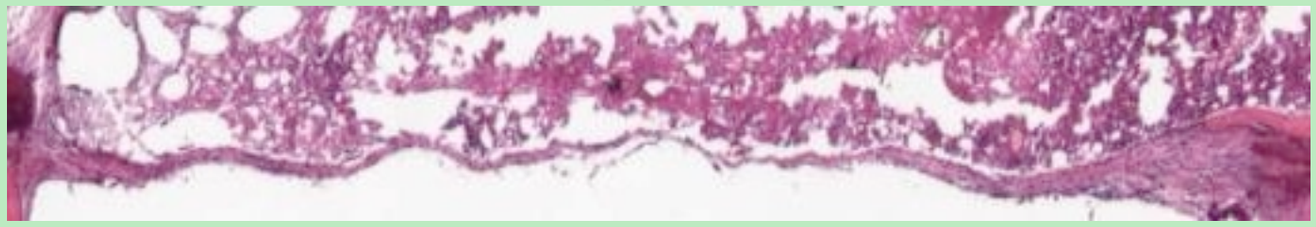
solidifed
calcium
phosphate
paste



A critical-sized round
craniotomy defect
(4 mm in diameter)

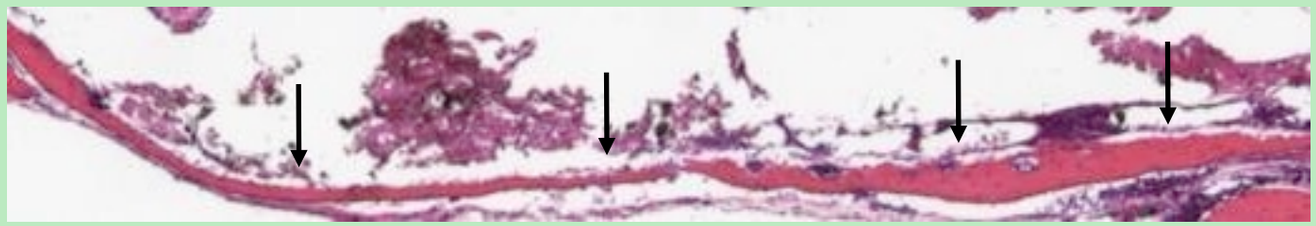
4 weeks

Control (GFP gene)

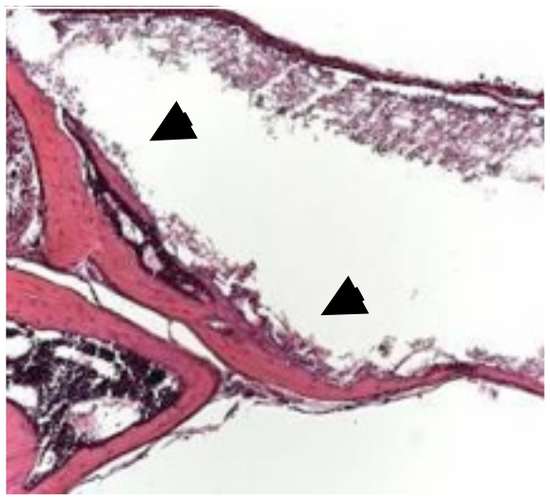


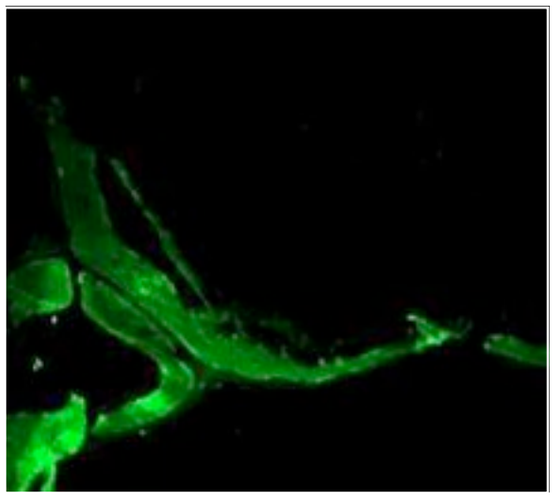
Adeno Virus

Polyplex Micelle



Immuno-staining of Type I collagen



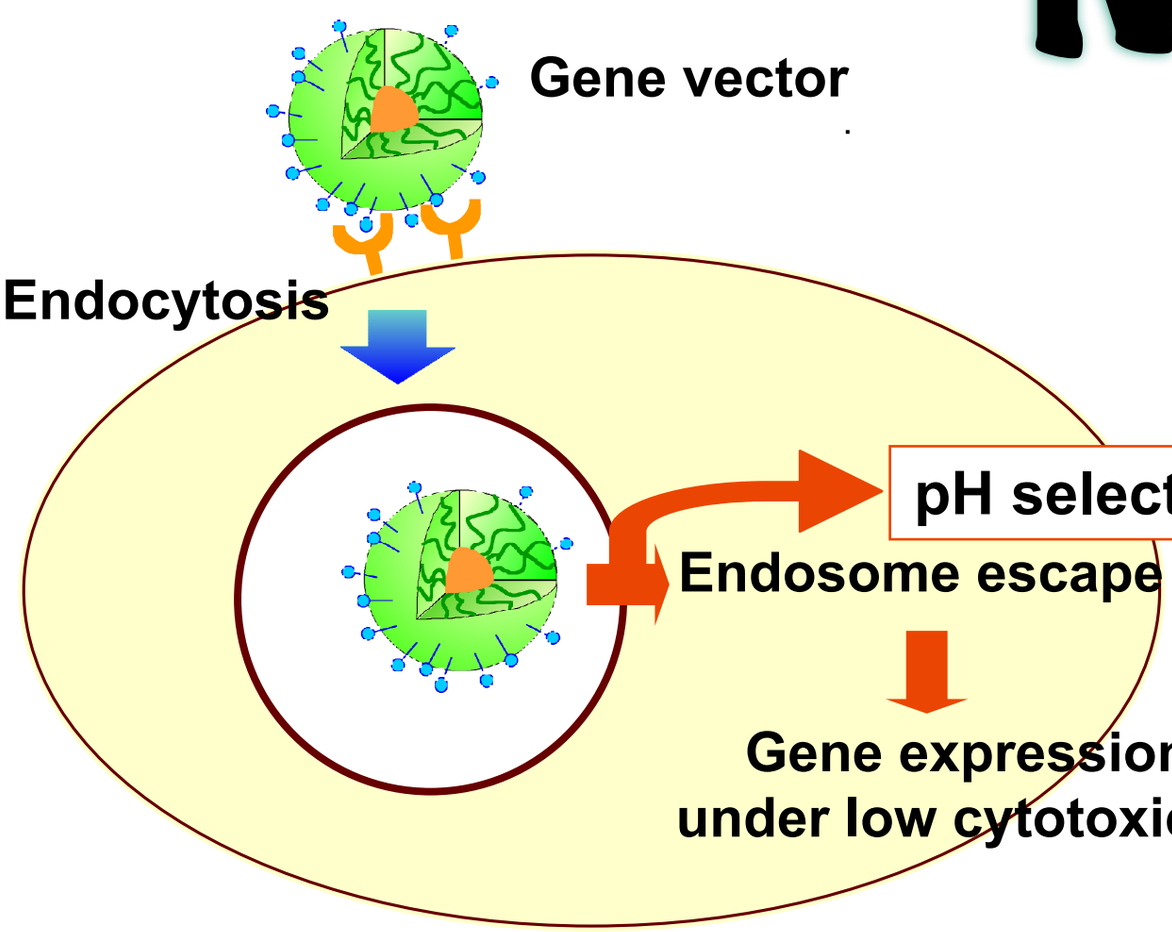


Polyplex micelles exceeded adeno virus to reveal substantial bone formation without inflammation

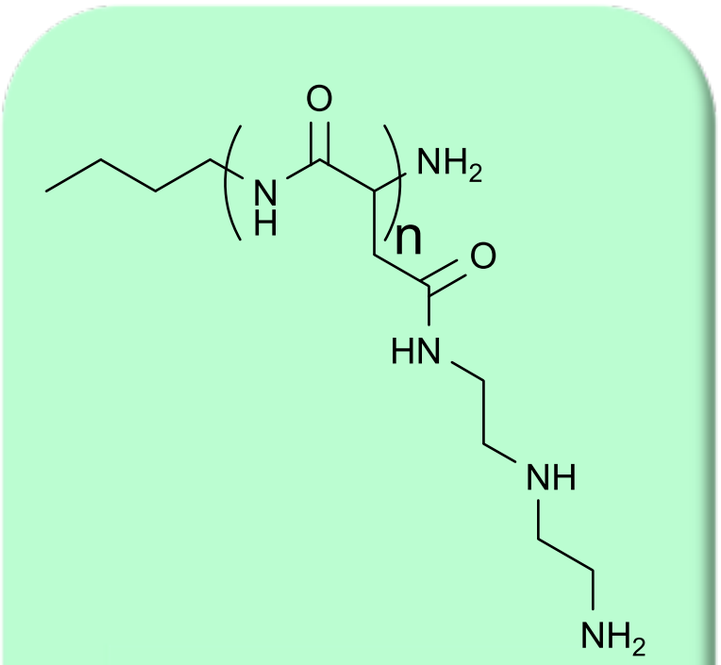
K. Itaka, et al, Molecular Therapy 15(9), 1655-1662 (2007)

Mechanism of Endosomal Escape

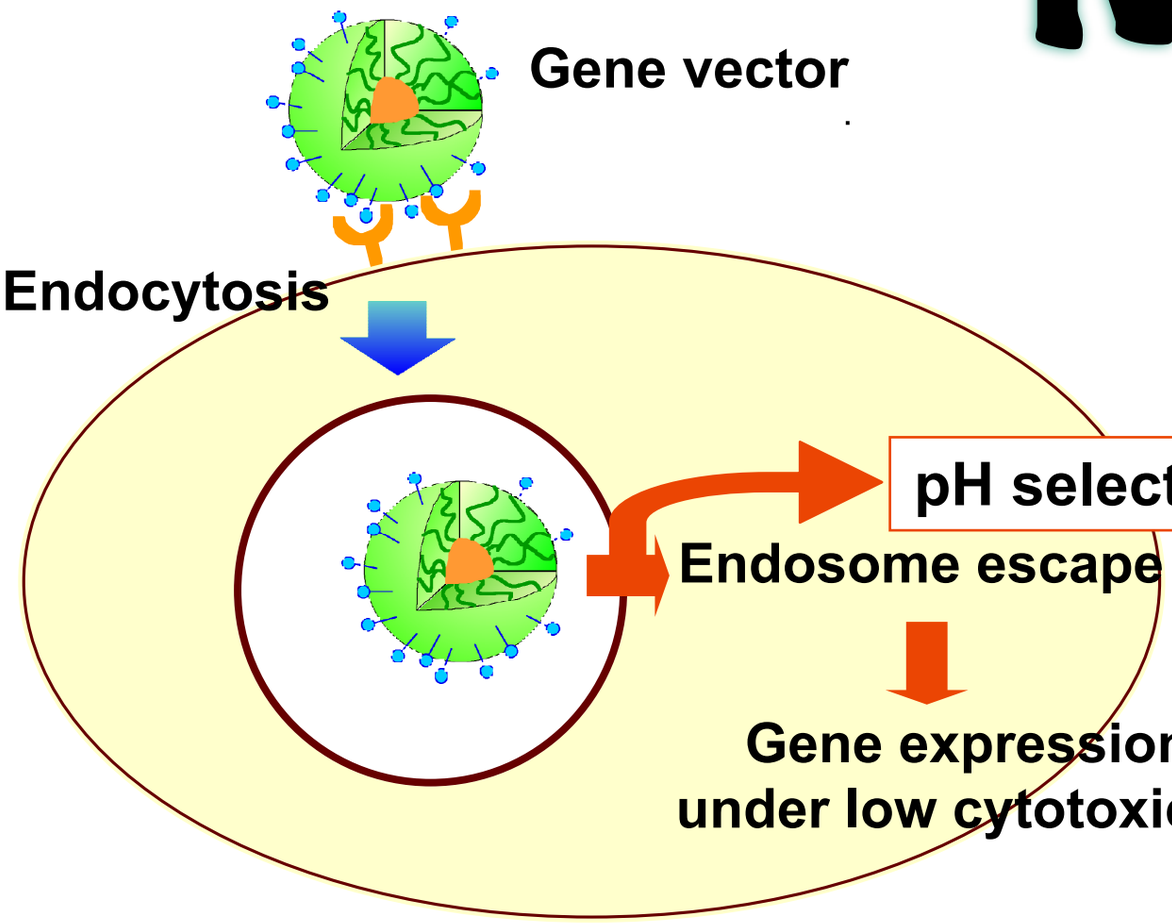
Mechanism?



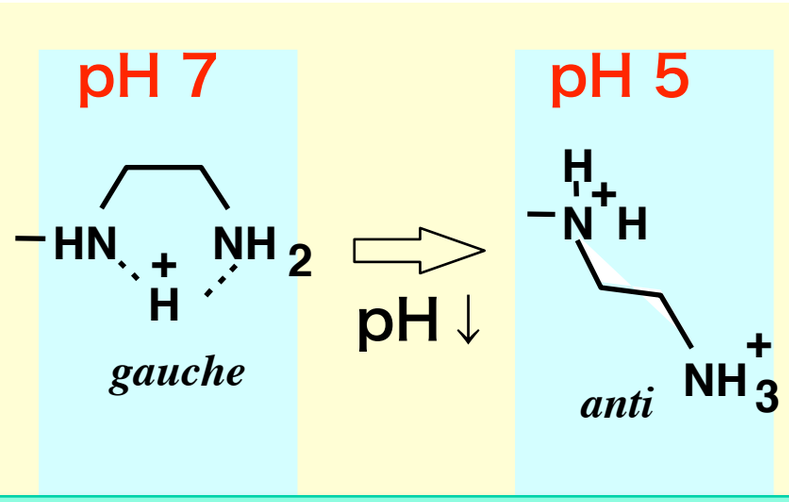
pH selective destabilization of endosomal membrane



Mechanism of Endosomal Escape

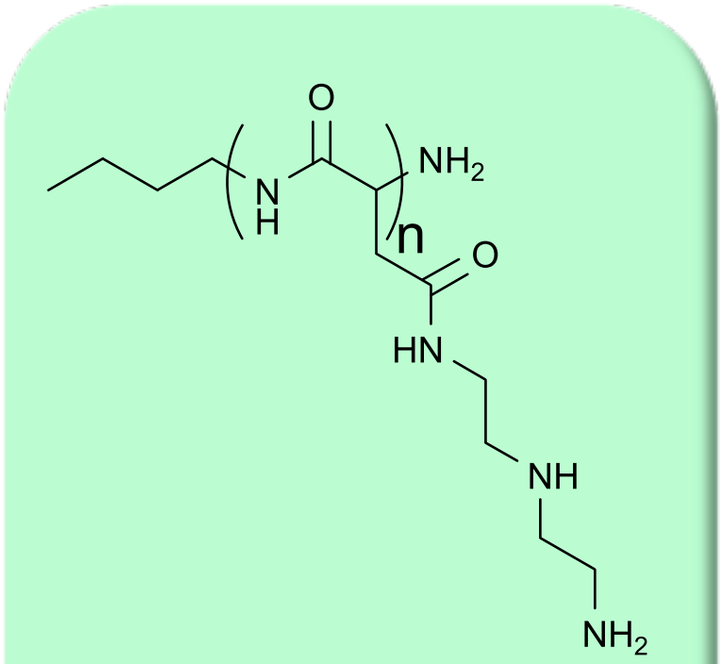


Me ?

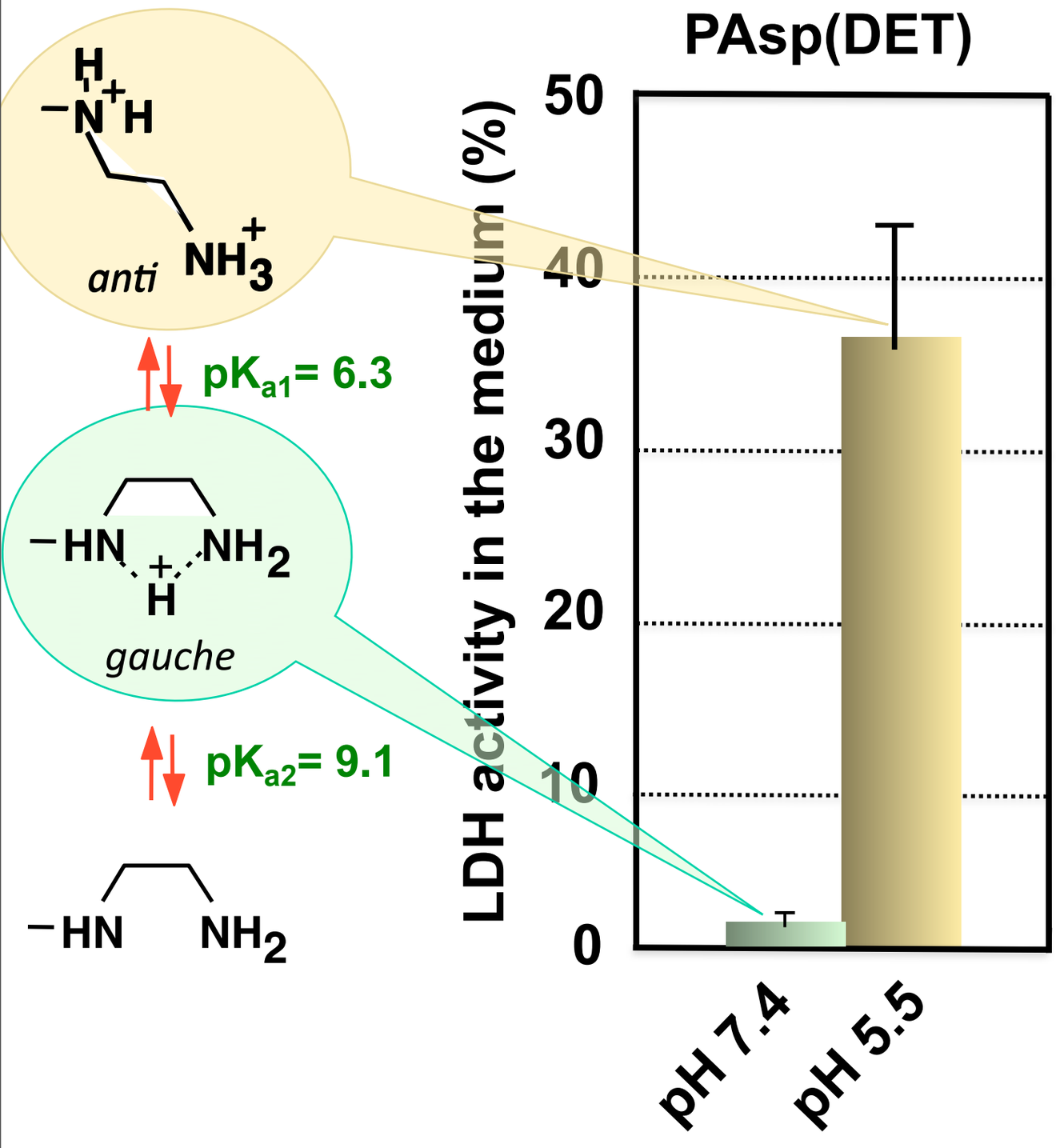


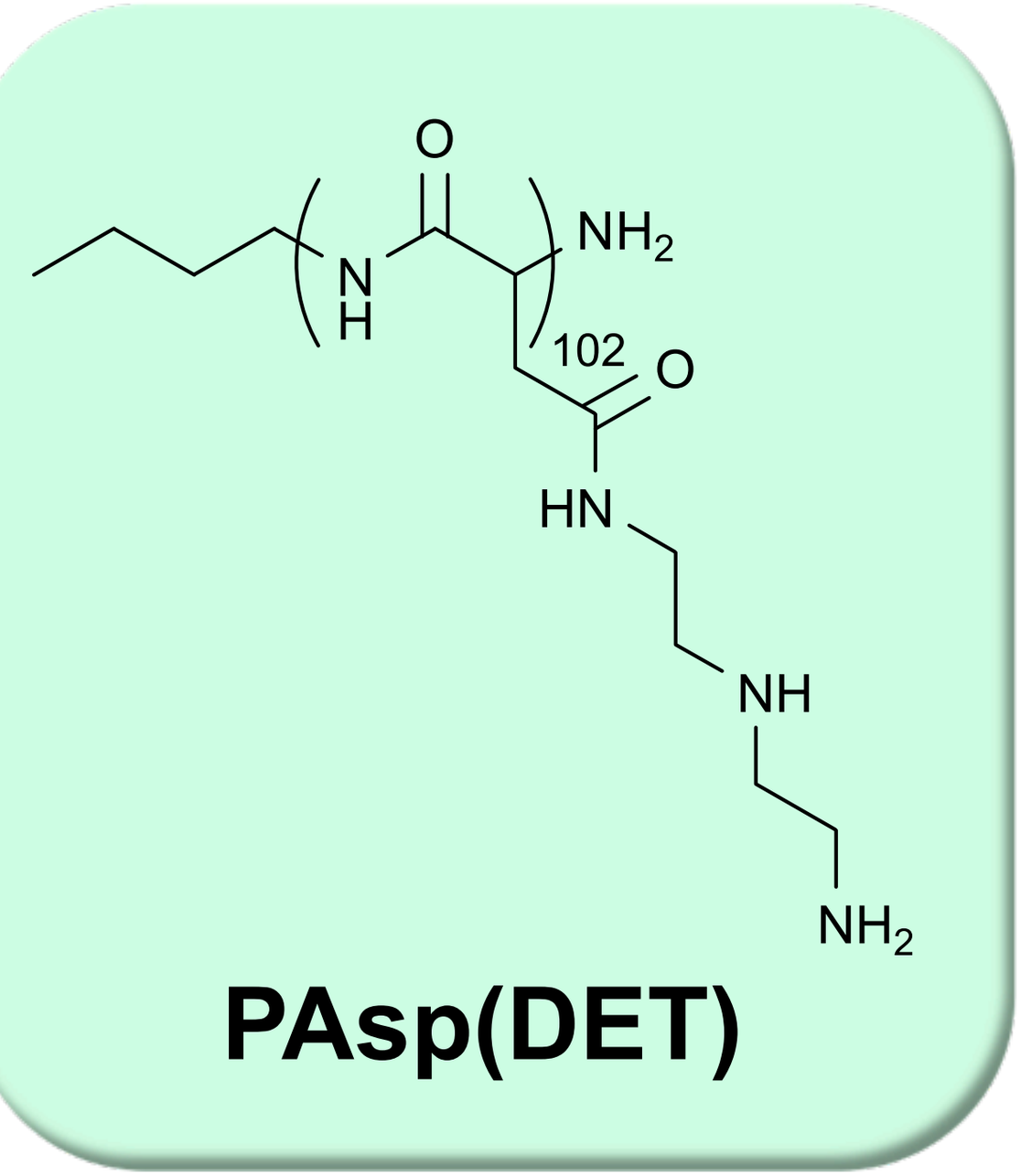
Membrane disruption induced by the protonation of diaminoethane units

pH selective destabilization of endosomal membrane



Destabilization of endosomal membrane: Assay by the leakage of cytoplasmic enzyme (LDH)



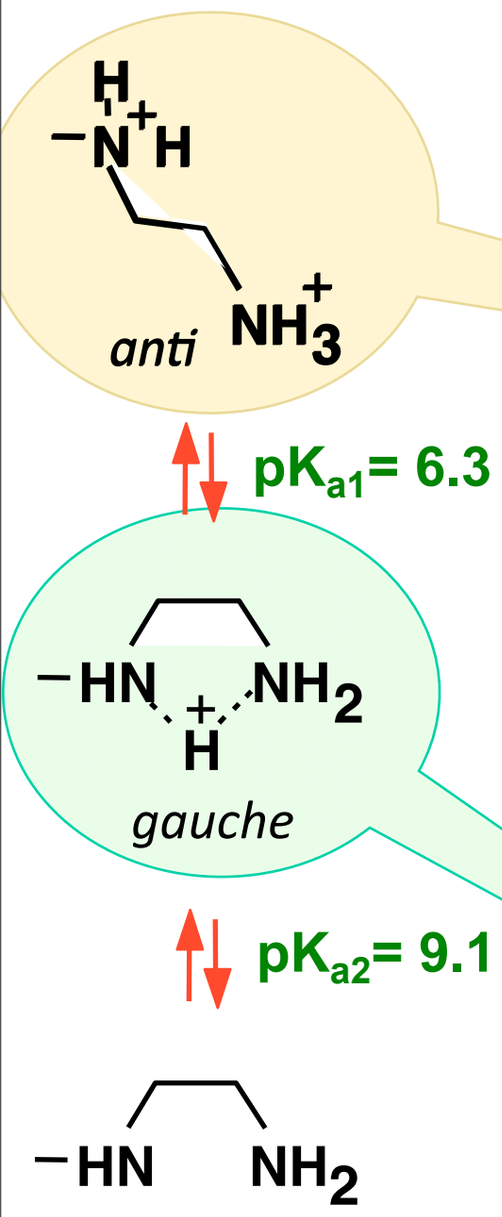


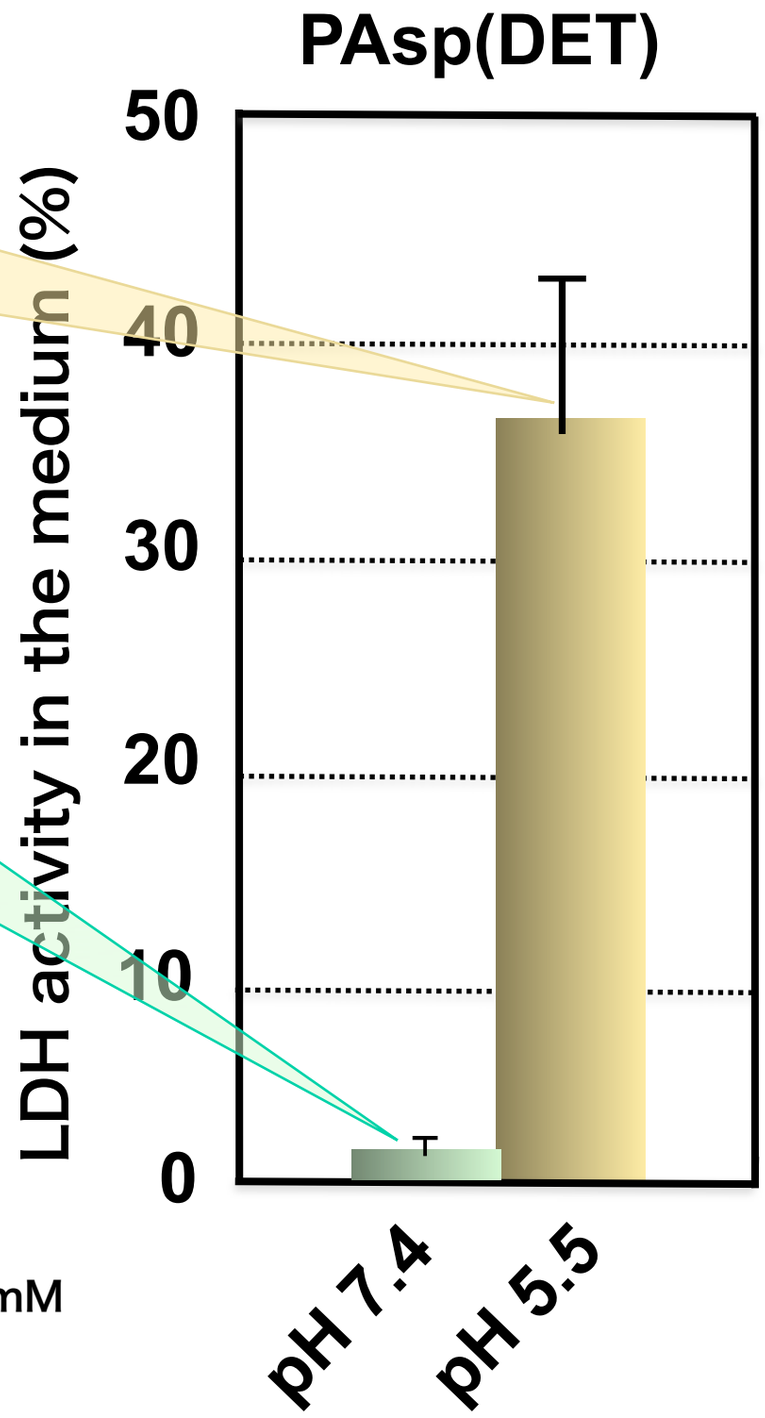
Amino group conc.:500 mM

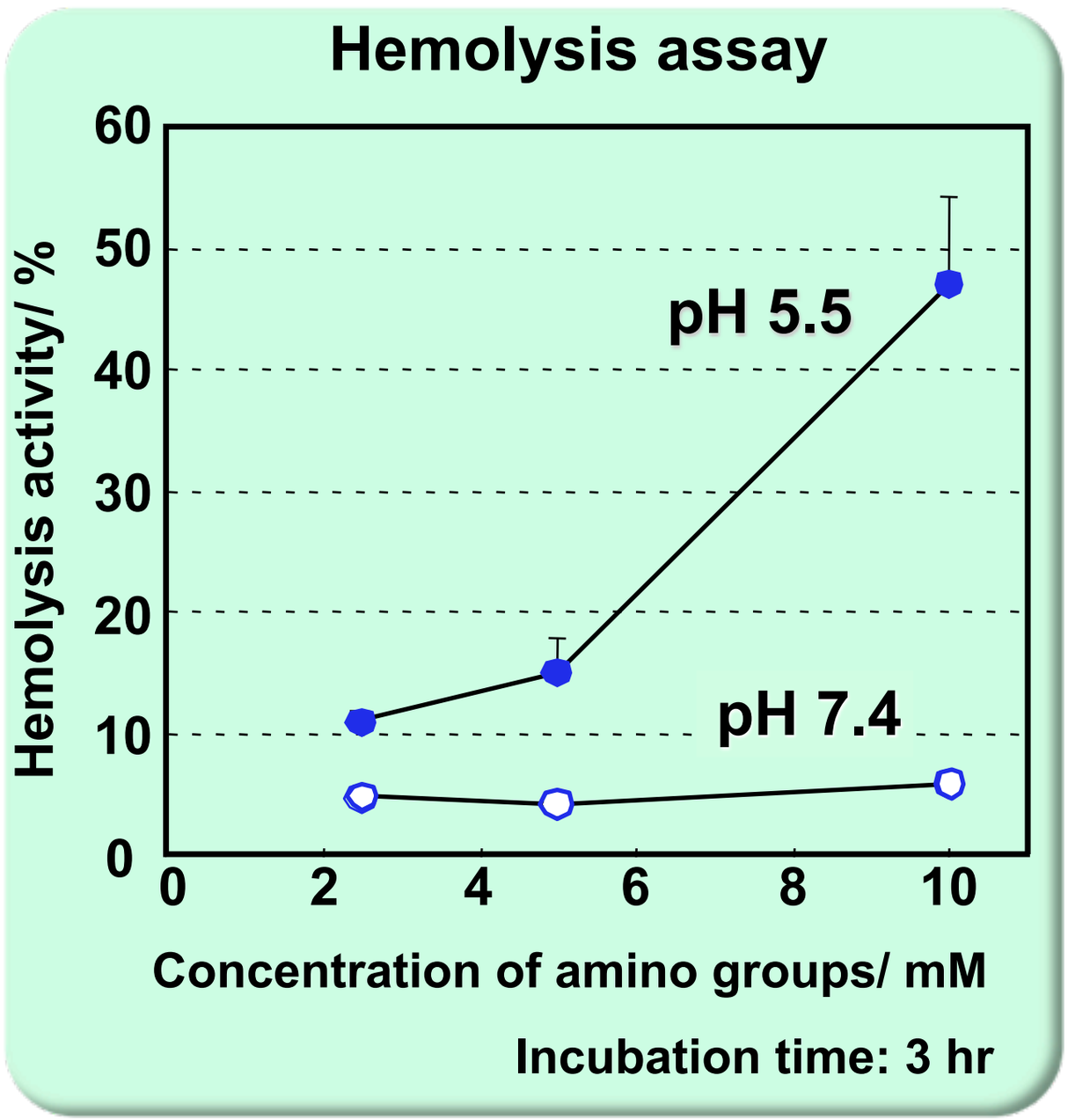
Cell：HUVEC

K. Miyata, et al, *J. Amer. Chem. Soc.*, in press

Destabilization of endosomal membrane: Hemolysis Assay



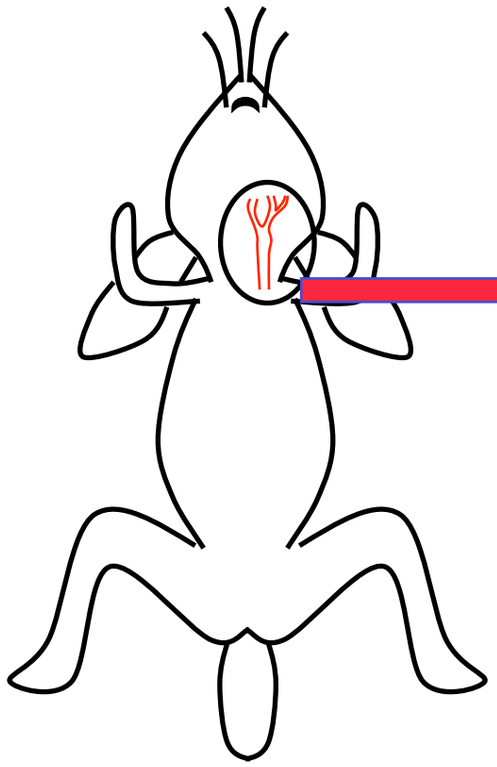


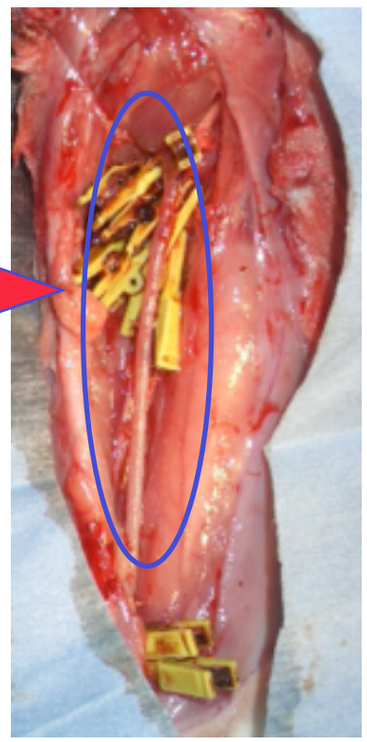


Amino group conc.:500 mM
Cell : HUVEC

PAsp(DET) exerts membrane destabilization selectively at pH 5.5 to facilitate endosomal escape of polyplex micelles

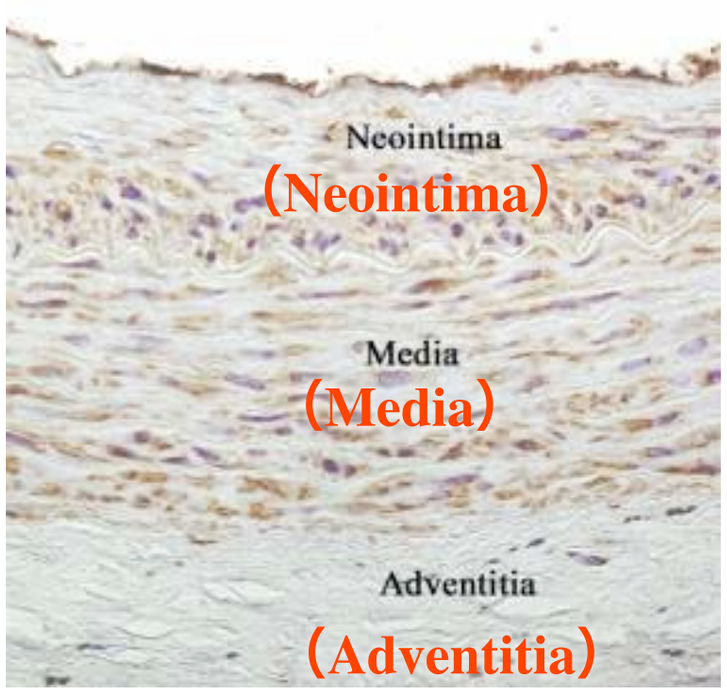
Gene expression to rabbit carotid artery by micellar nano-vector

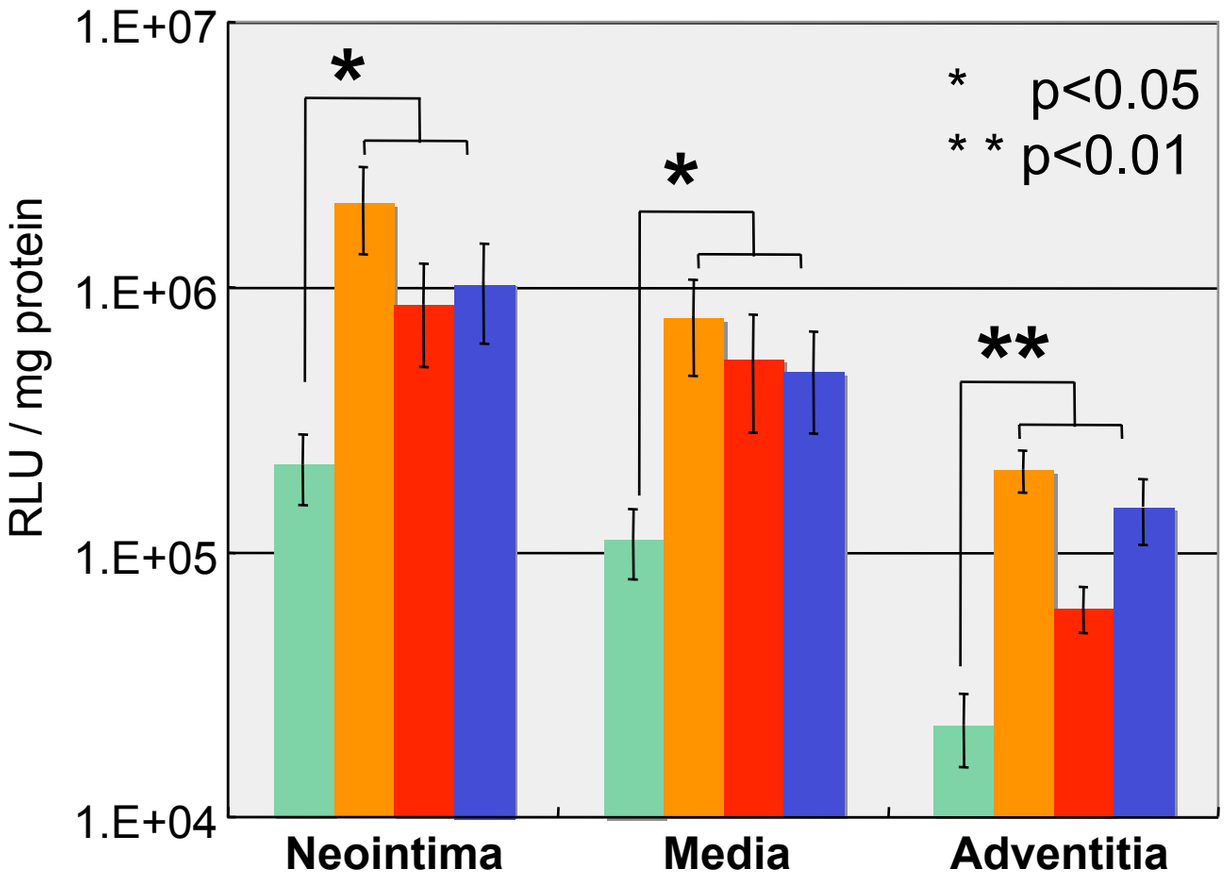




- Naked pDNA (**100% patency**)
- BPEI polyplex N/P10 (**62.5% patency**)
- P[Asp(DET)] polyplex N/P 40 (**50% patency**)
- PEG-*b*-P[Asp(DET)] micelle N/P 40 (**100% patency**)

FLAG tag gene expression

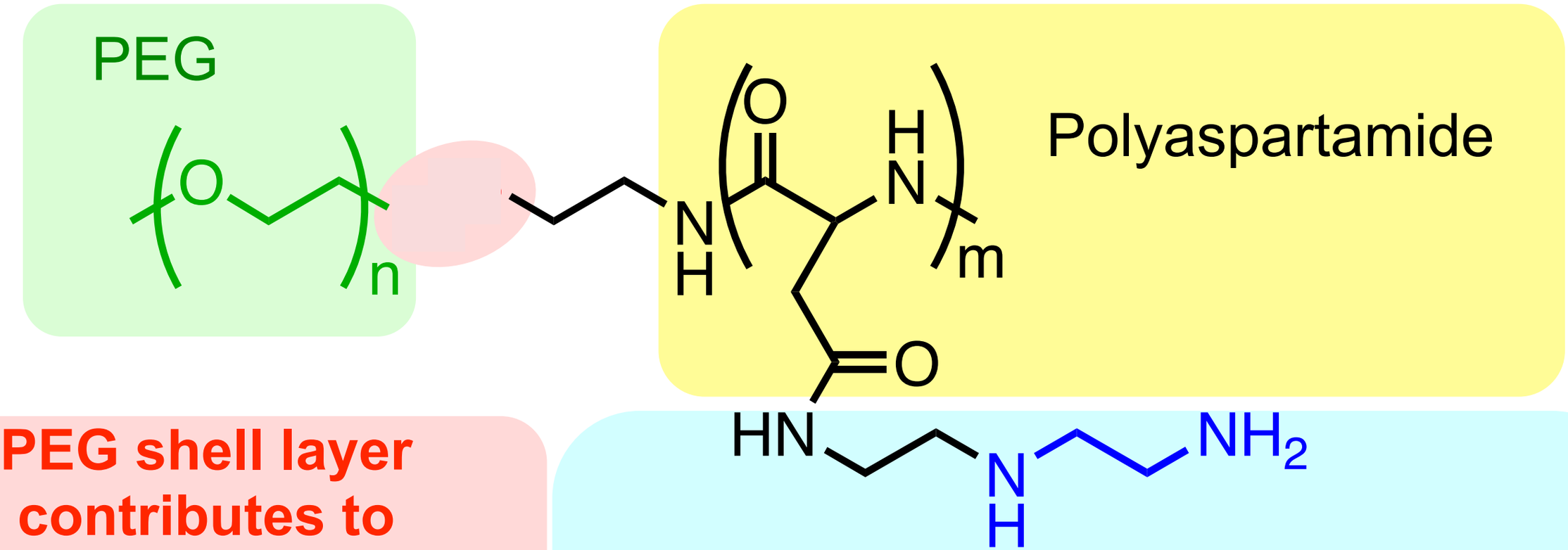




Micellar nanovector achieved efficient gene transfer to carotid artery with neointimal hyperplasia without any vascular occlusion by intravascular method

D. Akagi, et al, *Gene Ther.*, 14, 1029-1038 (2007)

Polymer Design for PEG-Detachable micelle



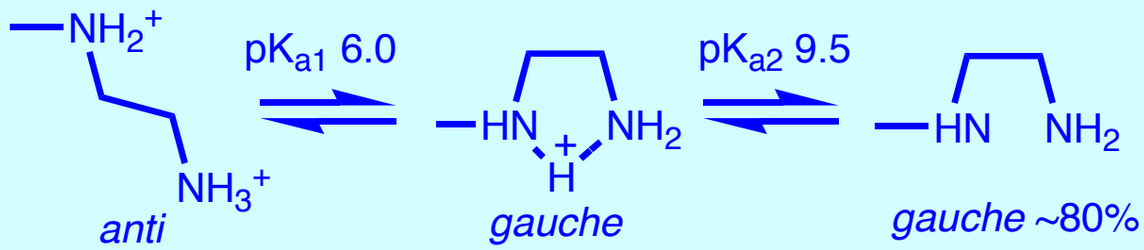
PEG shell layer contributes to biocompatibility and colloidal stability of polyplex micelles

BUT

Often reducing transfection efficacy due to steric effect

<Ethylenediamine unit>

The two step protonation permits DNA condensation at pH 7.4 (mono-protonated) and high membrane disruptive ability in endosomal pH (di-protonated) to induce endosomal escape.



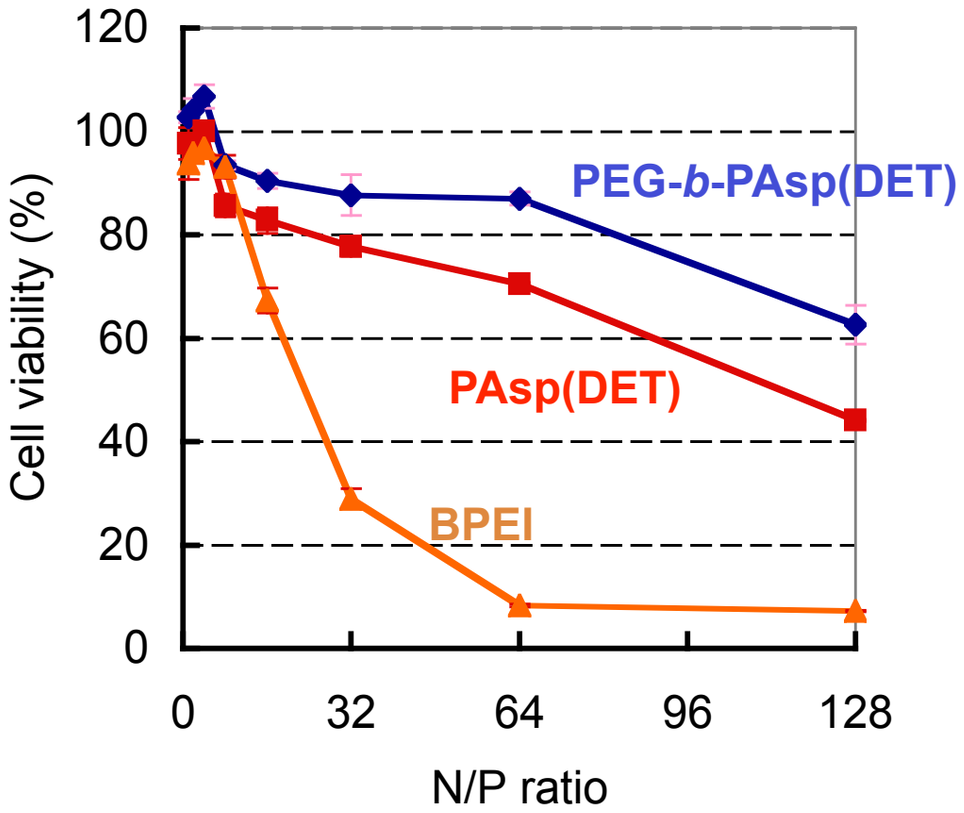
Kanayama, et al *ChemMedChem*, **2006**, 1, 439-434

Transfection efficiency and cytotoxicity against vascular smooth muscle cells (SMC)

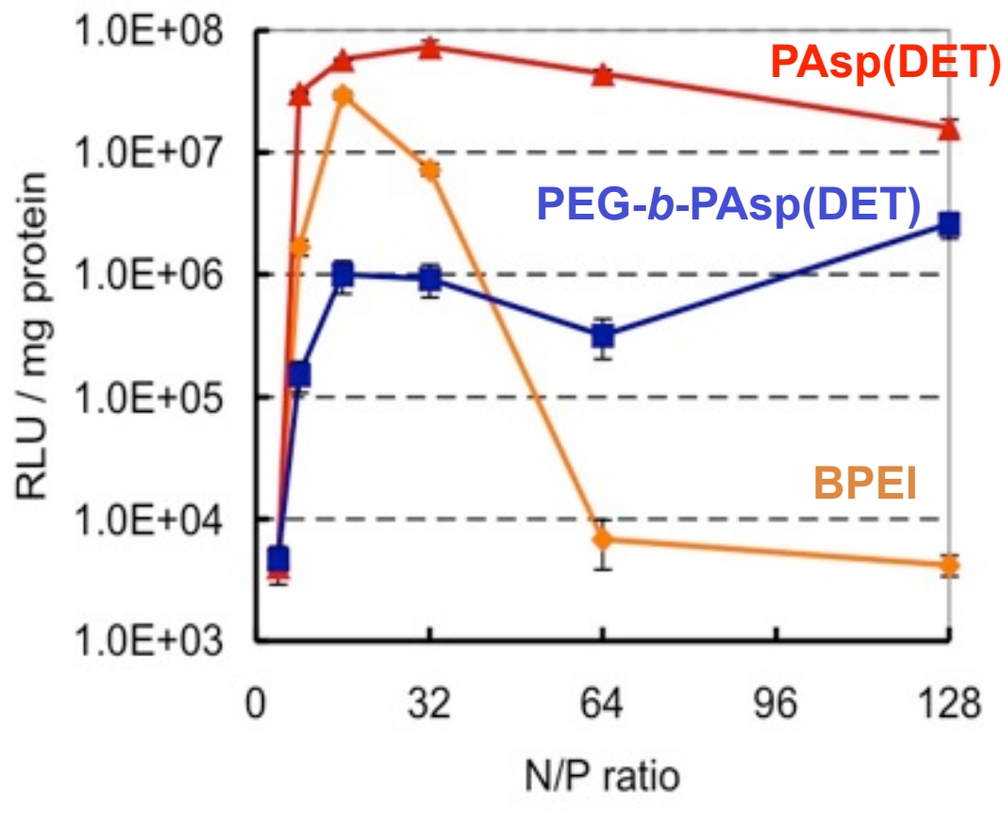
PAsp(DET)(98mer): polycation without PEG block and forming polyplex with plasmid DNA

BPEI: branched polyethyleneimine (*Mw*=25KDa)

Cytotoxicity assessment (MTT assay)



Transfection efficinecy (luciferase assay)



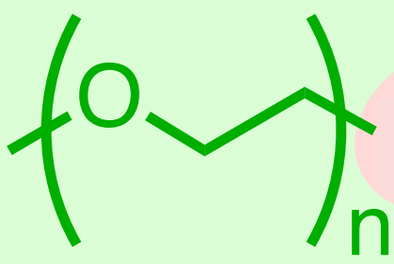
Lower cytotoxicity of PEG-*b*-PAsp(DET) and PAsp(DET) than BPEI.

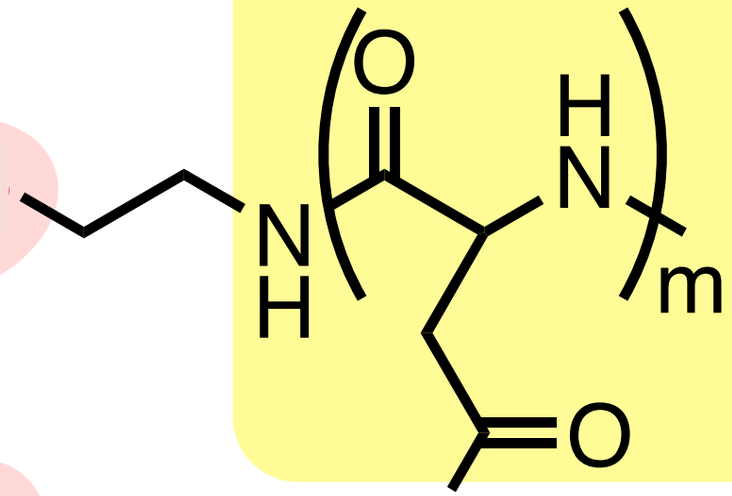
Higher transfection efficiency of PAsp (DET) and BPEI polyplexes than PEG-*b*-PAsp(DET) micelles.

D. Akagi, et al, *Gene Ther.*, 14, 1029-1038 (2007)

Polymer Design for PEG-Detachable micelle

PEG





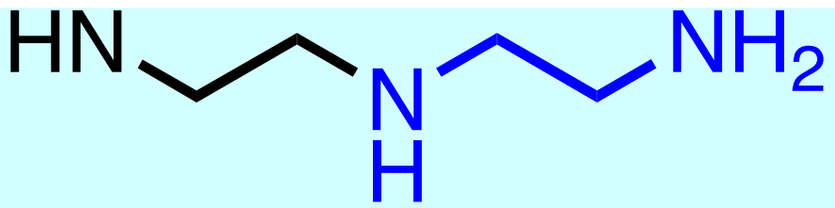
Polyaspartamide

Disulfide linkage
(SS linkage)

Cleavable under intracellular reduced environment

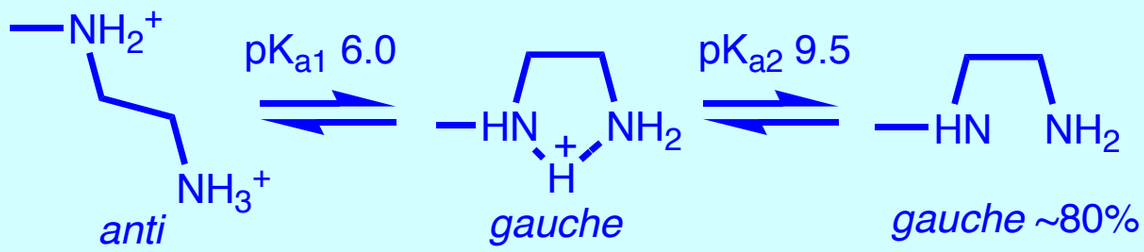
PEG-SS-P[Asp(DET)]

S. Takae, et al, *JACS*, 130(18) 6001-6009 (2008)



<Ethylenediamine unit>

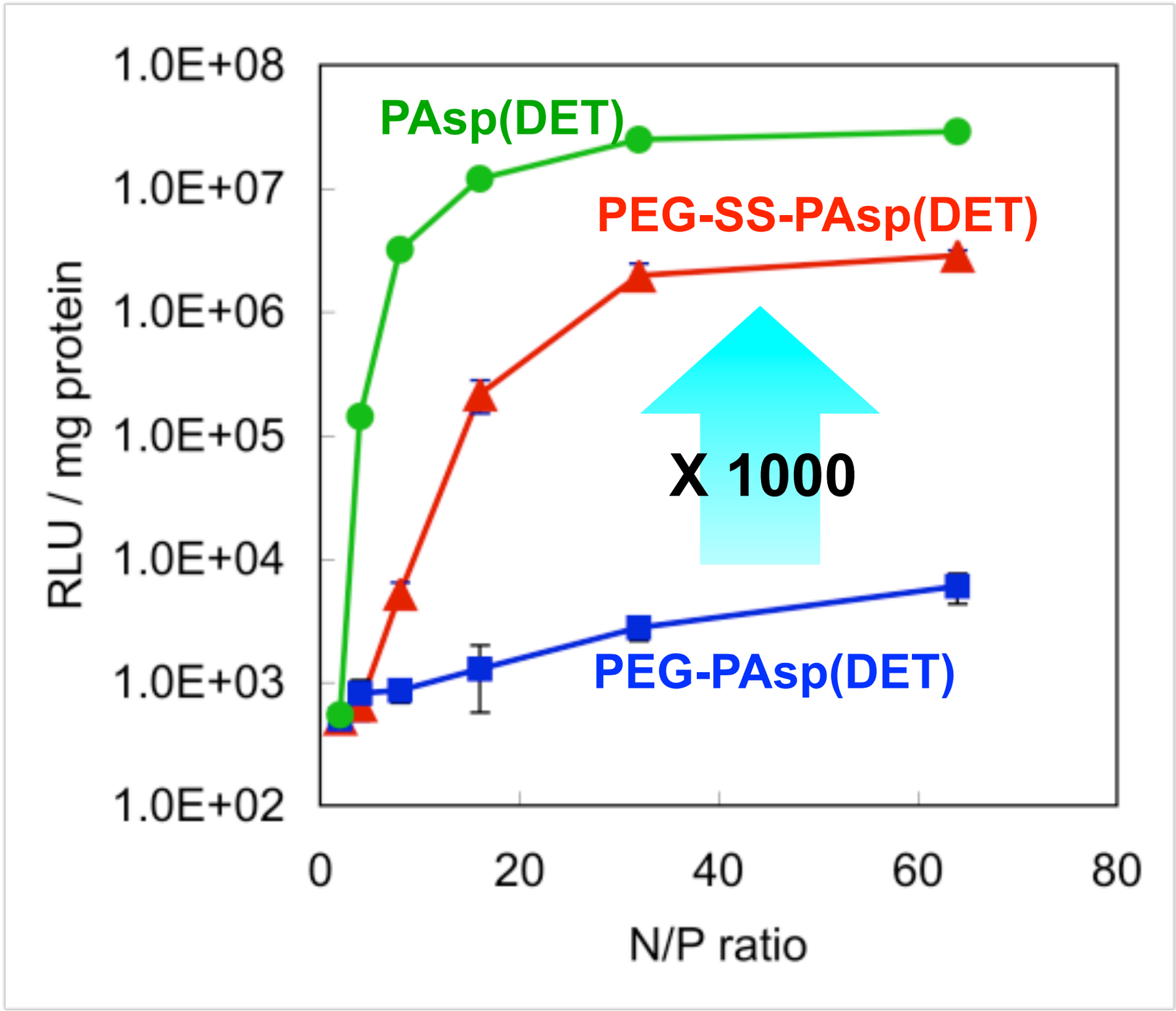
The two step protonation permits DNA condensation at pH 7.4 (mono-protonated) and high membrane disruptive ability in endosomal pH (di-protonated) to induce endosomal escape.



Kanayama, et al *ChemMedChem*, **2006**, 1, 439-434

Transfection by PEG-detachable polyplex micelles

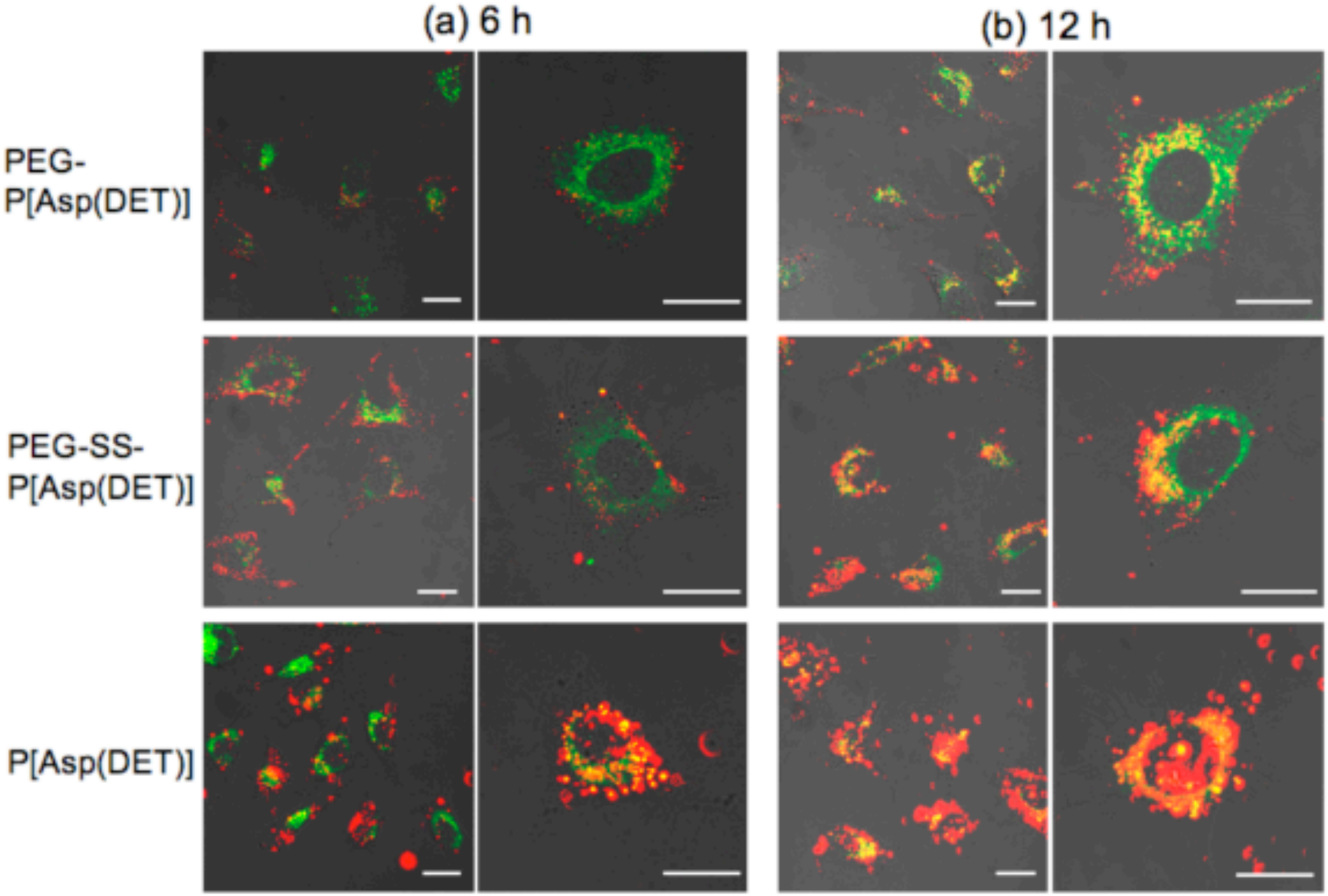
Luciferase assay (HeLa cells)



S. Takae, et al, *JACS*, 130(18) 6001-6009 (2008)

Transfection by PEG-detachable polyplex micelles

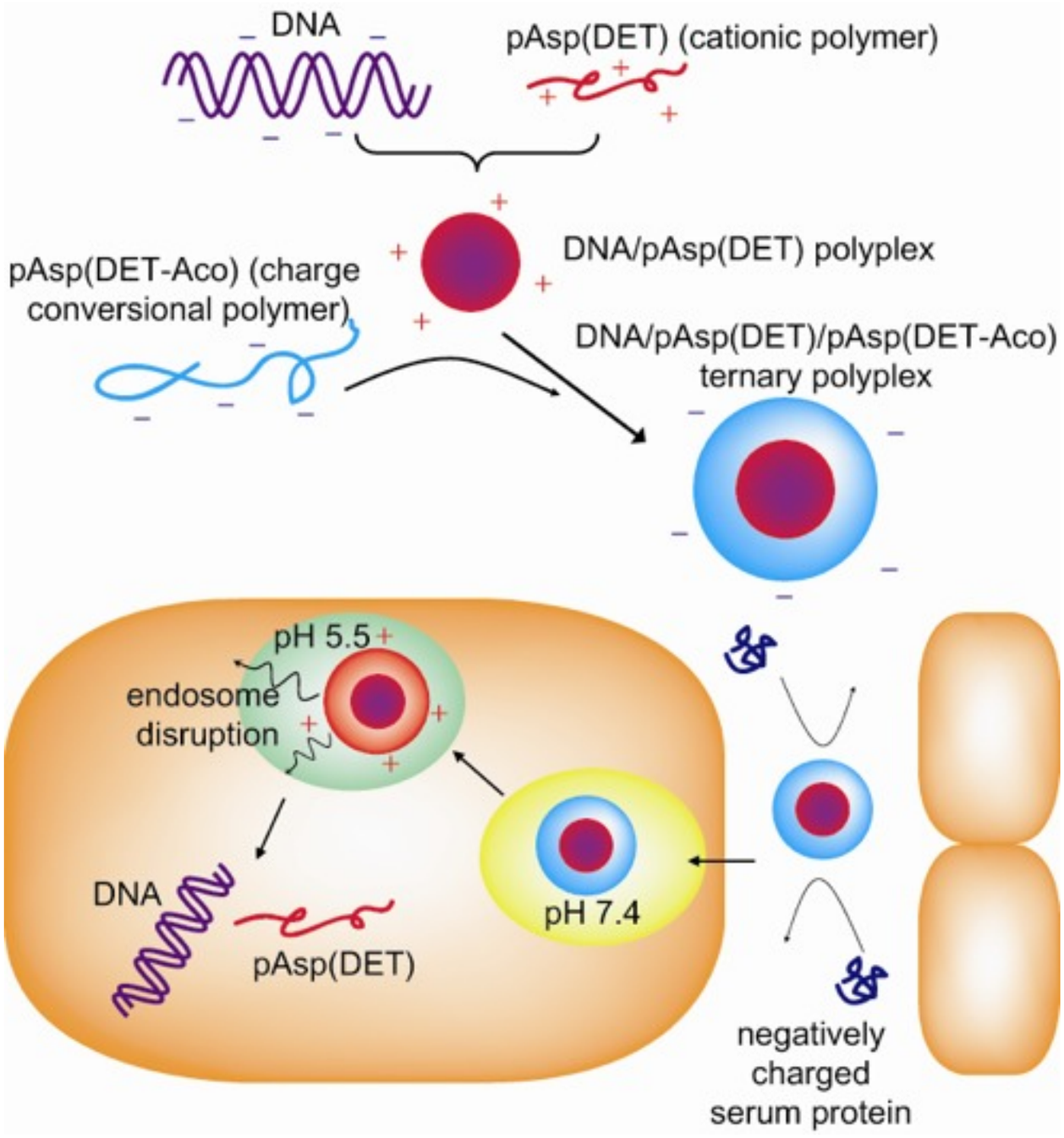
Time dependent change in intracellular distribution of pDNA



Cy5-labeled pDNA (Red), Lysotracker (Green)

S. Takae, et al, *JACS*, 130(18) 6001-6009 (2008)

Charge-conversional polyplex system

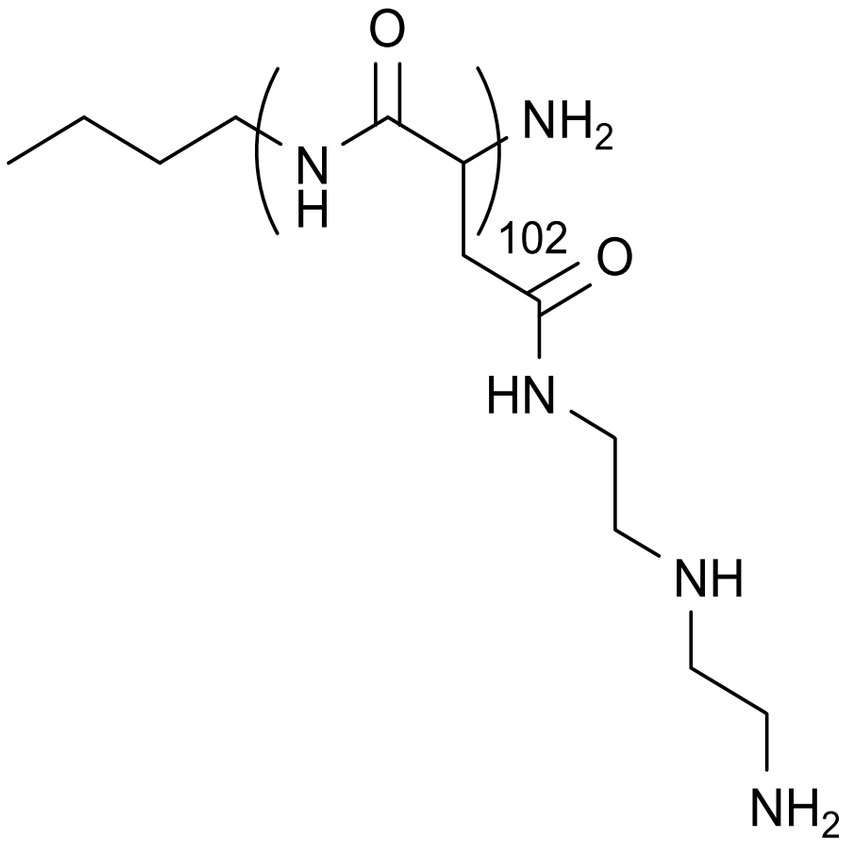


If the polyplex had negatively charged in the cell exterior, it could reduce the toxicity.

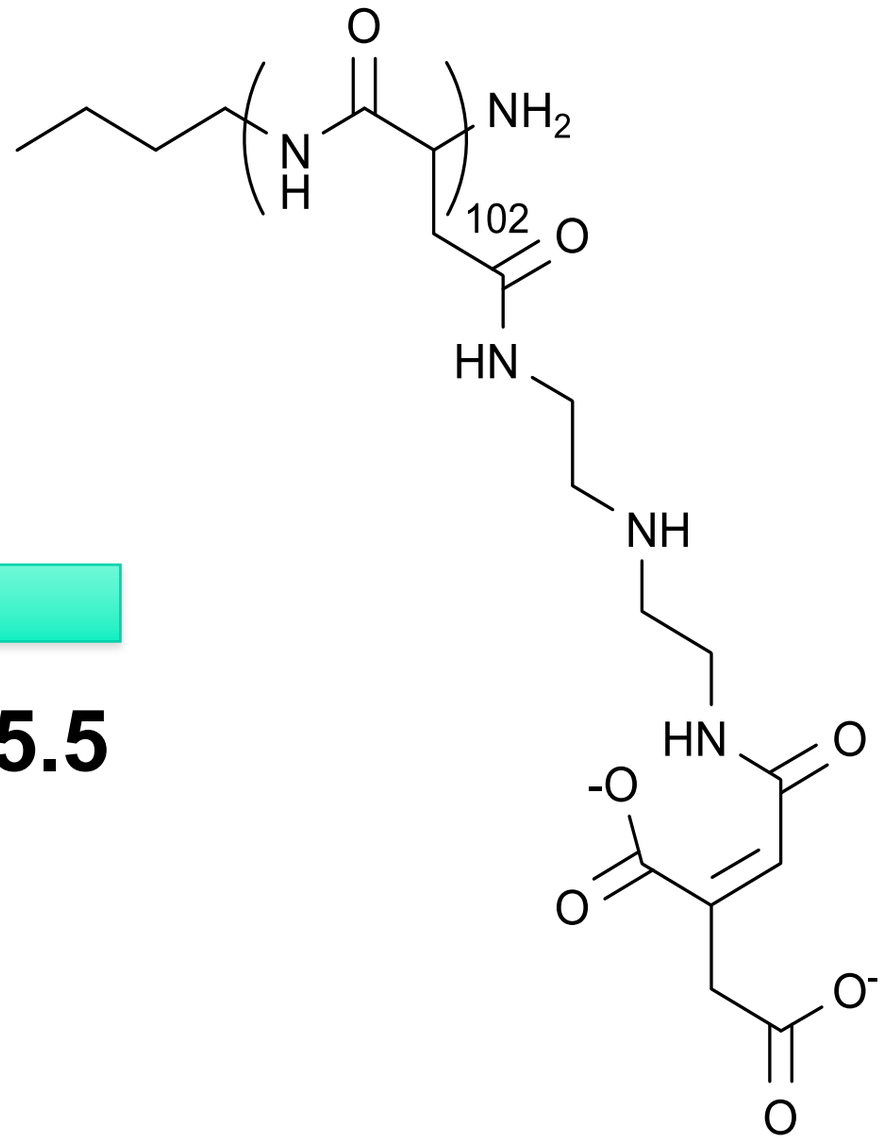
If the charge of the polyplex turned to positive after internalization into the cell, it could disrupt endosome effectively.

Y. Lee, et al, *JACS* 129, 5362-5363 (2007); *Angew. Chem., Int'l Ed.*, 47,5163-5166 (2008)

pAsp(DET)/pAsp(DET-Aco)



pH < 5.5

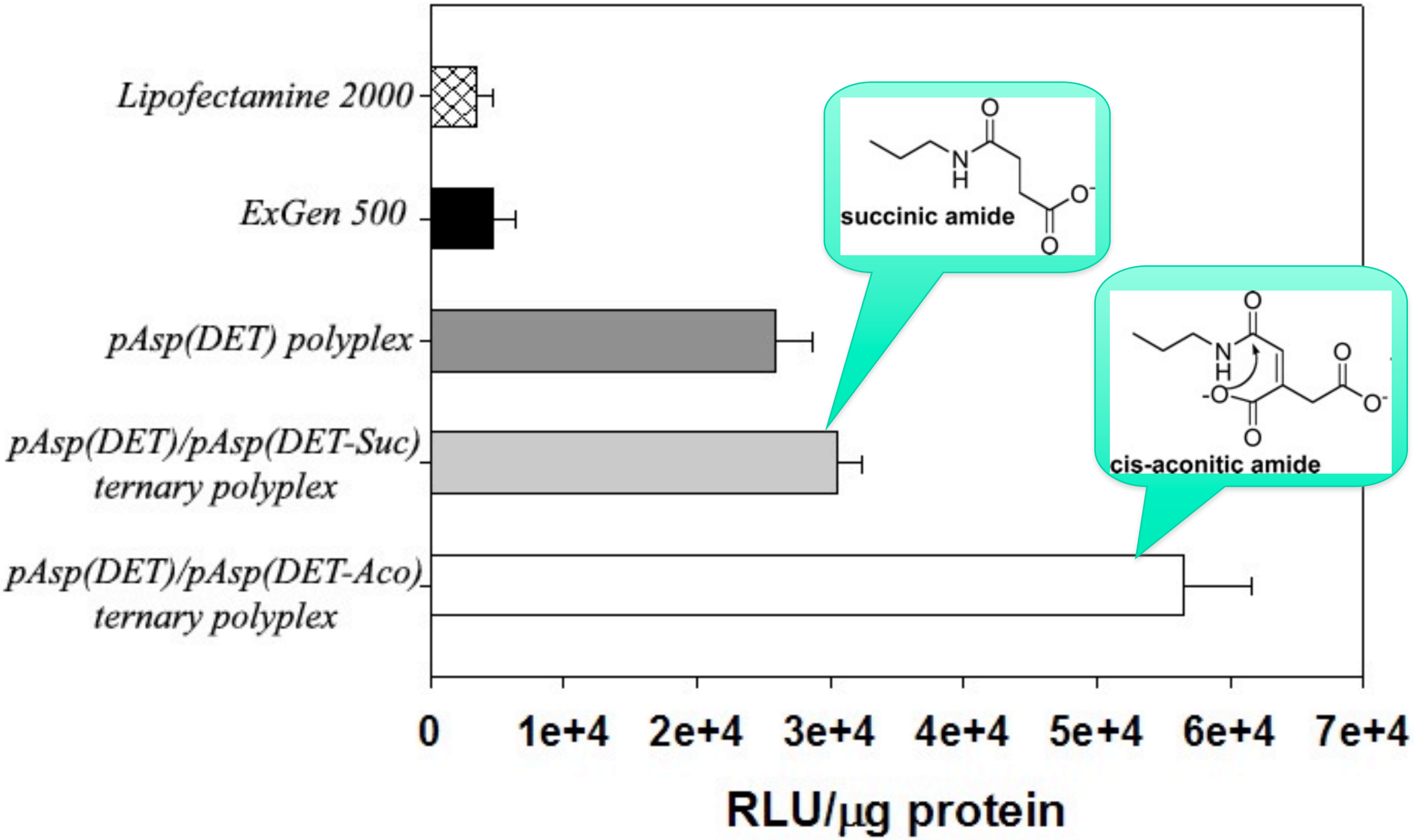


pAsp(DET) : cationic polymer with endosome disruption moiety

pAsp(DET-Aco) : charge-conversional polymer

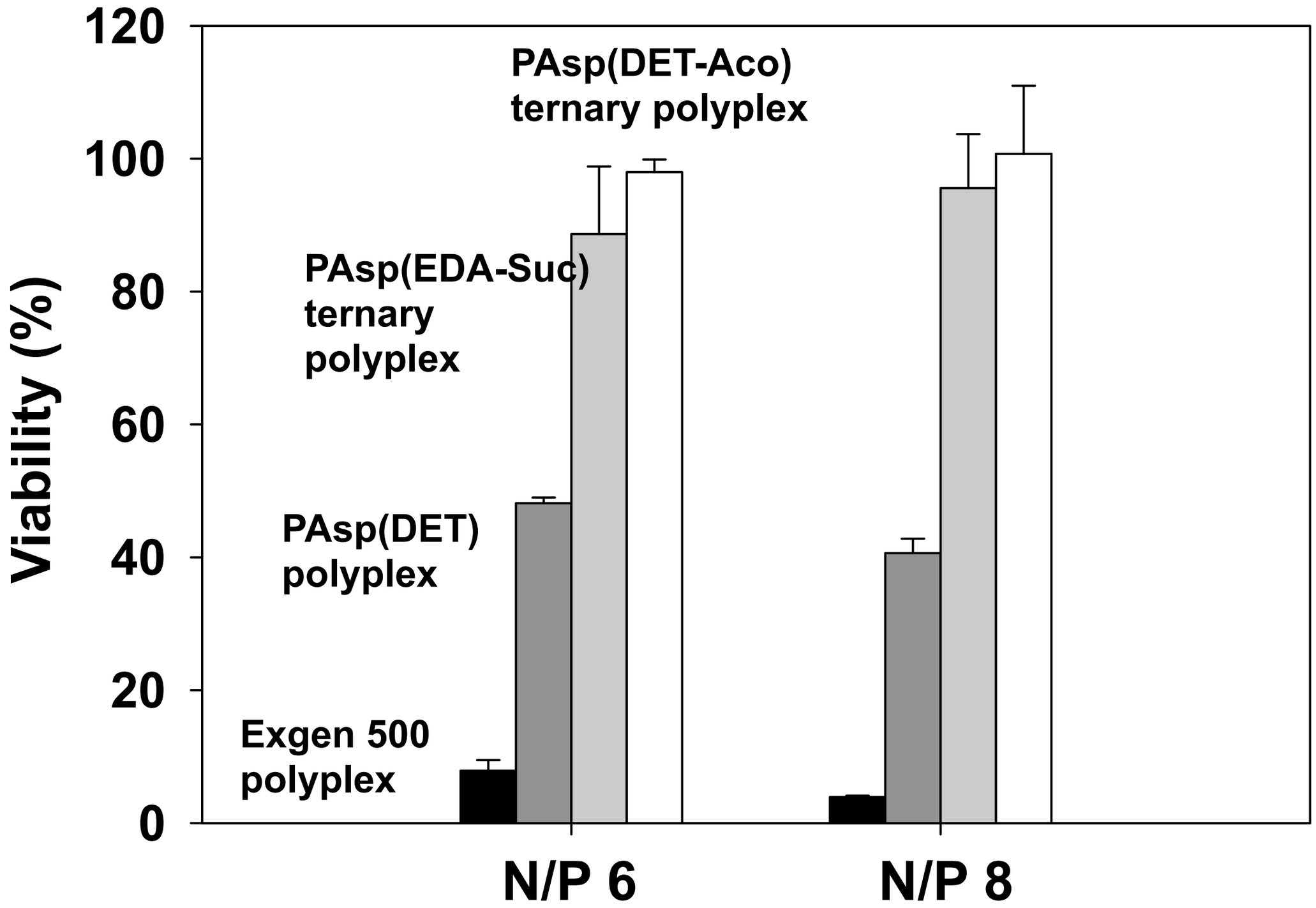
Y. Lee, et al, *Angew. Chem., Int'l Ed.*, 47(28) 5163-5166 (2008)

Transfection efficiency of ternary polyplex on HUVEC



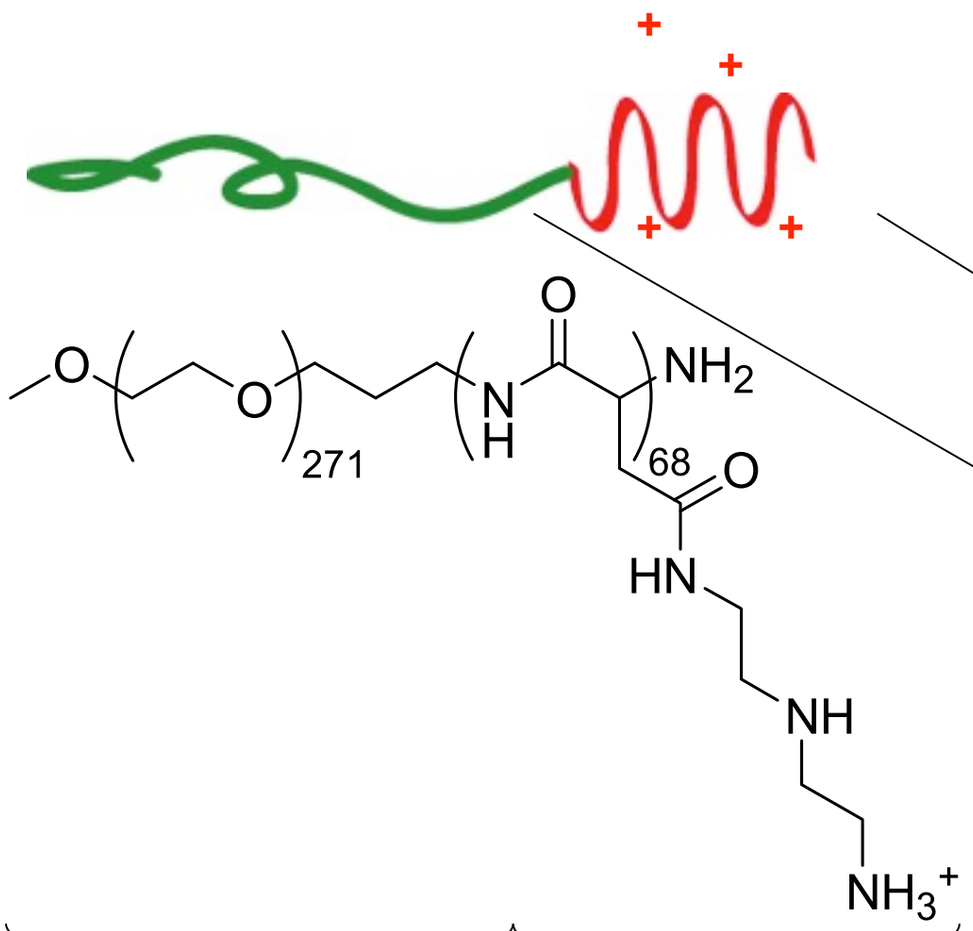
*HUVEC (Human umbilical vein endothelial cells) are difficult to be transfected and sensitve to toxicity.
*The charge-conversional ternary polyplex (white) showed high transfection efficiency in this primary cells.

Cytotoxicity of ternary polyplex against HUVEC



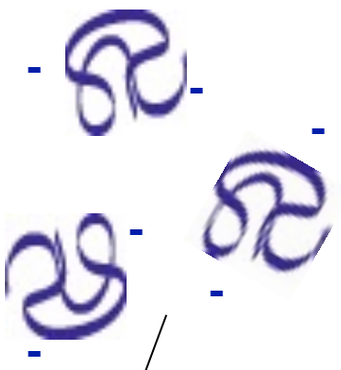
Y. Lee, et al, *Angew. Chem., Int'l Ed.*, 47(28) 5163-5166 (2008)

PIC micelles:
an efficient protein delivery system into cytoplasm



Polyethyleneglycol (PEG): Neutral block

Ionic block with endosomal escaping property (PAsp (DET))



Counterions: protein

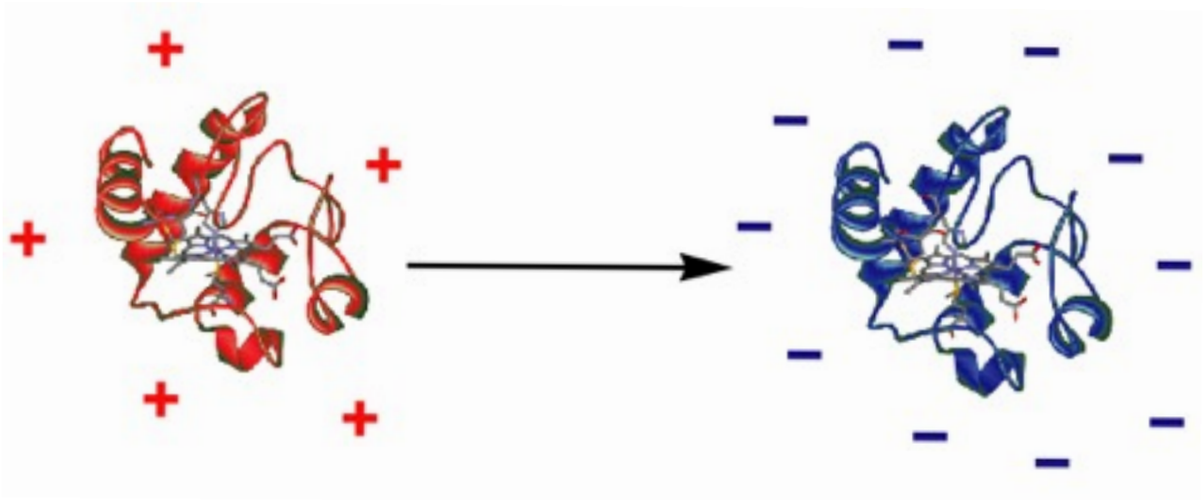
core

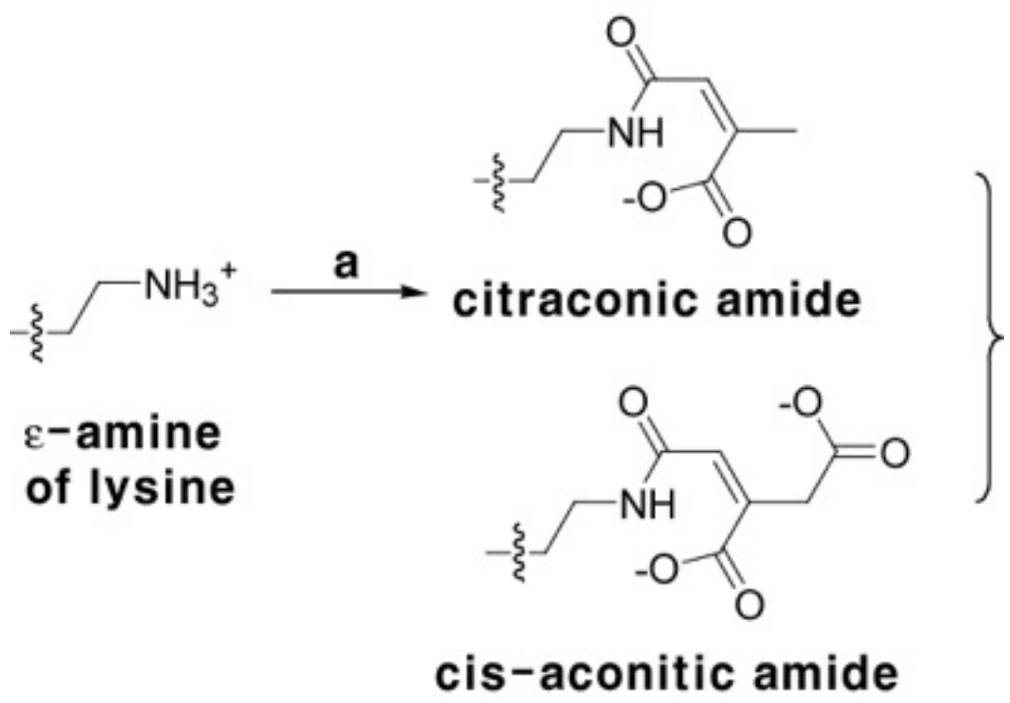
shell

Polyionic complex (PIC) micelles for protein nanocarriers!!

Y. Lee, et al, *J. Am. Chem. Soc.* 129(17), 5362-5363 (2007); *Angew. Chem. Int. Ed.* 47(28), 5163-5166 (2008)

Charge-conversional modification of protein (Cytochrome C)



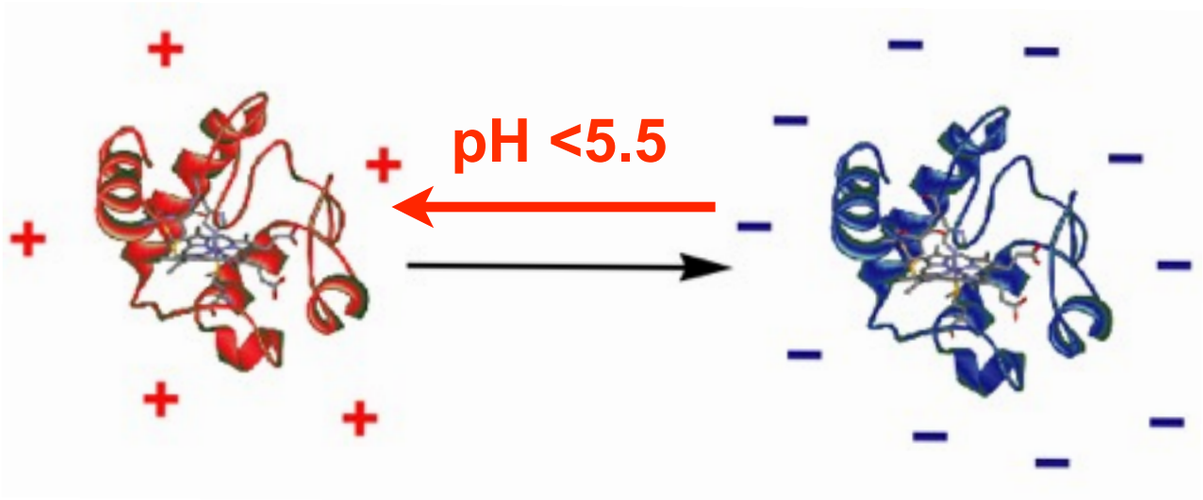


a: citraconic anhydride or cis-aconitic anhydride

Protein	Charge density (Da/charge)
CytC	+1391
Cyt-Cit	-501
Cyt-Aco	-320

Charge-density can be increased by the charge-conversion!

Charge-conversional modification of protein (Cytochrome C)



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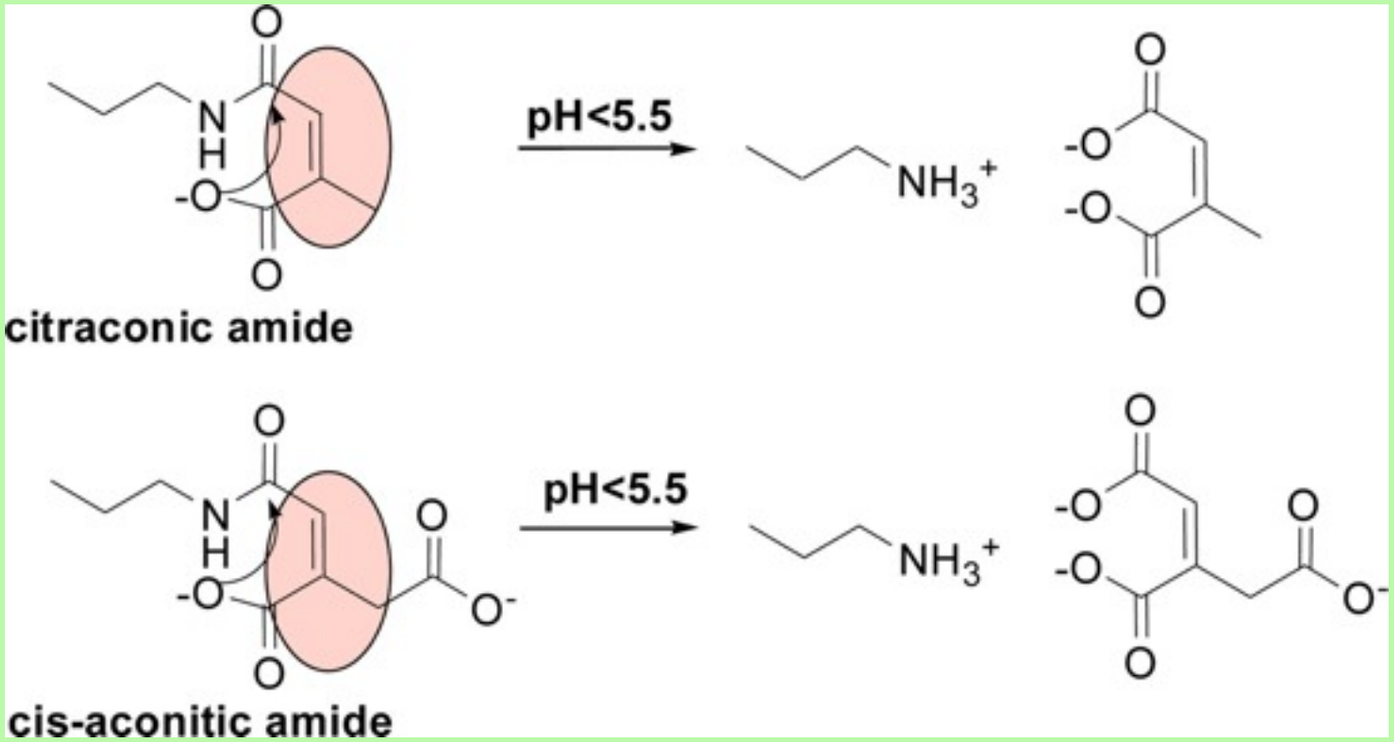
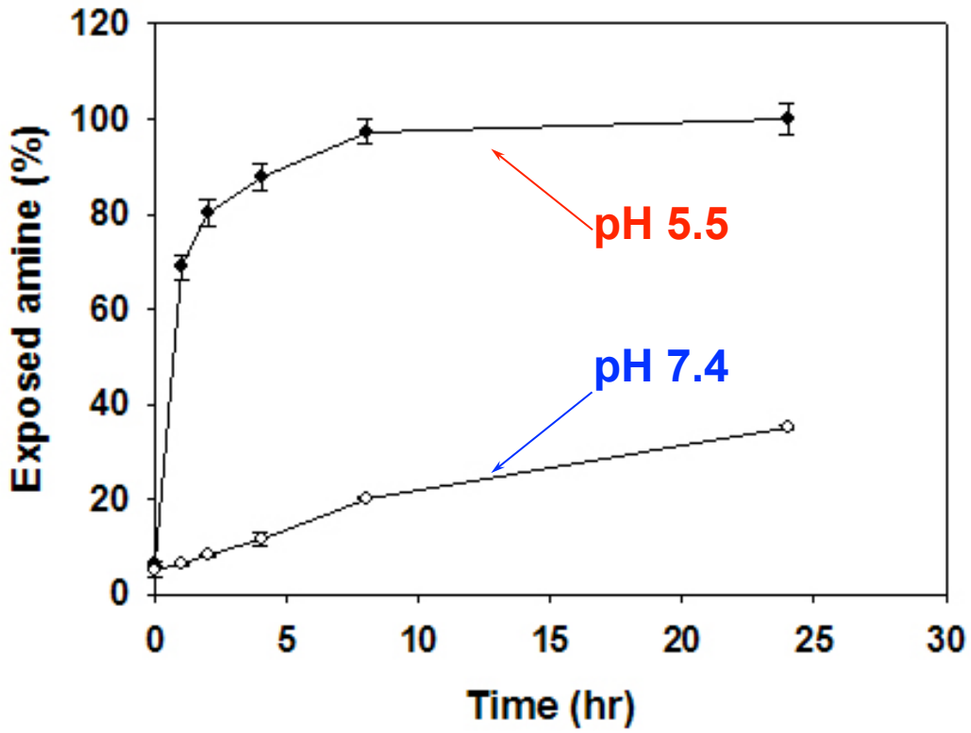


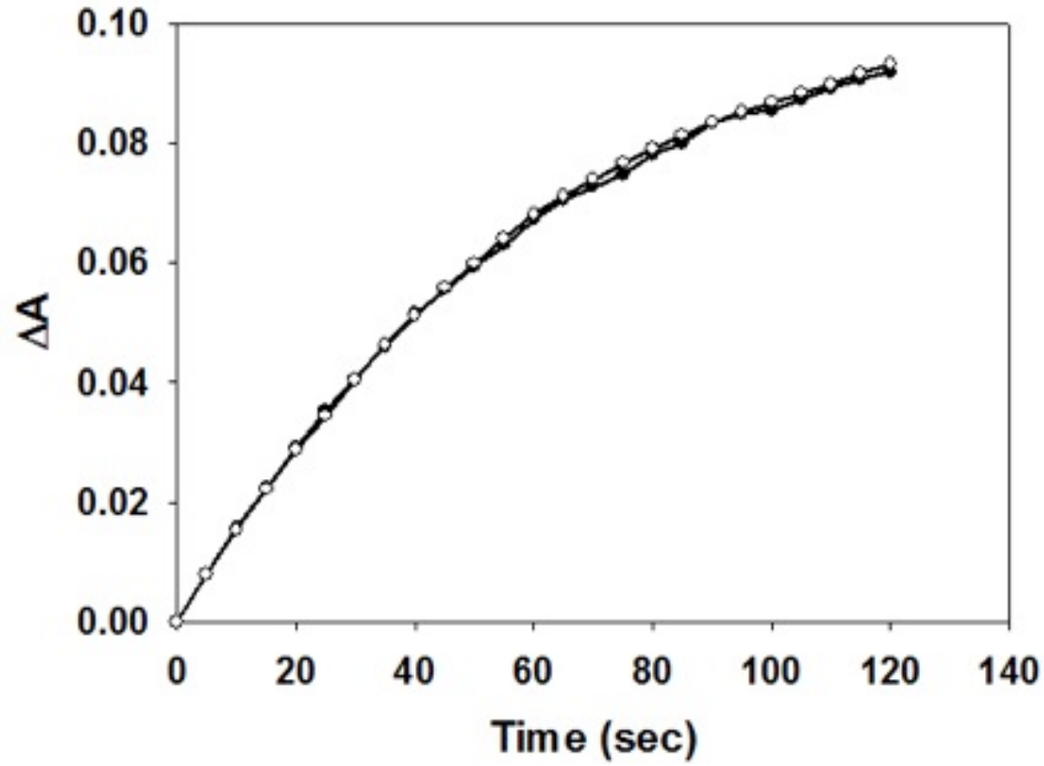
Charge-density can be increased by the charge-conversion!

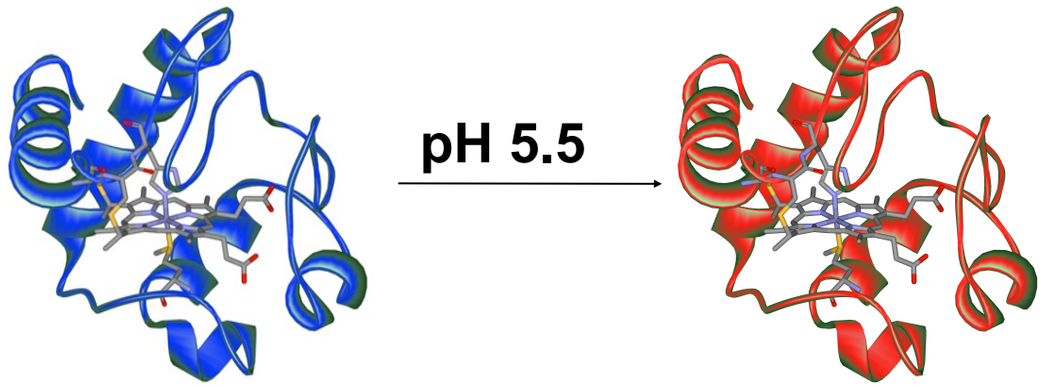
Reversibility of the charge-conversion

pH-sensitive reversal



protein activity

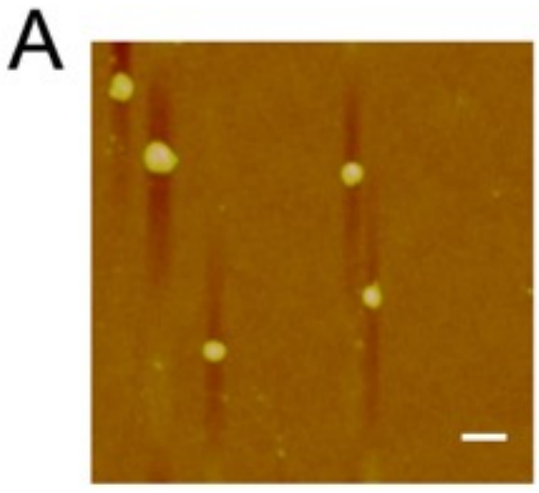


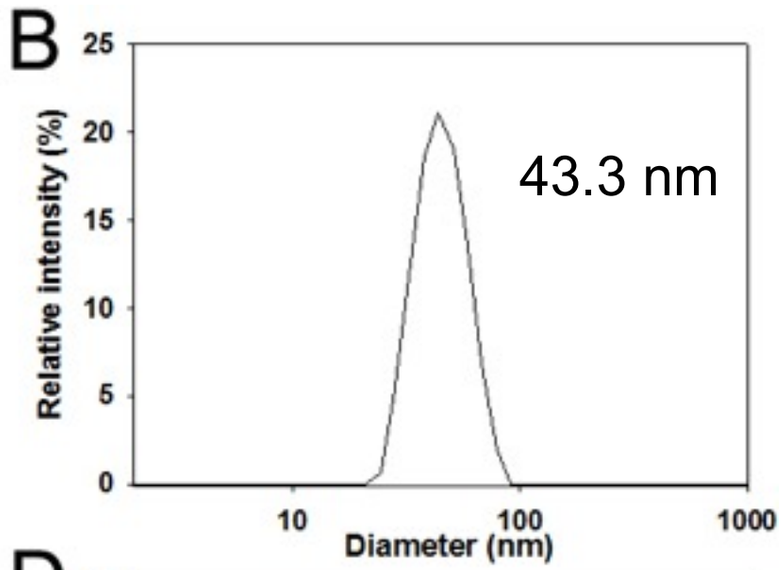


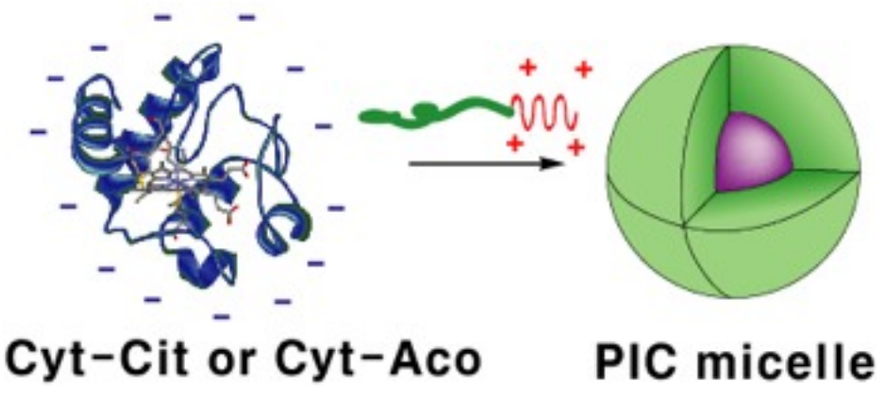
The charge-conversion can be reversed rapidly at endosomal pH 5.5.

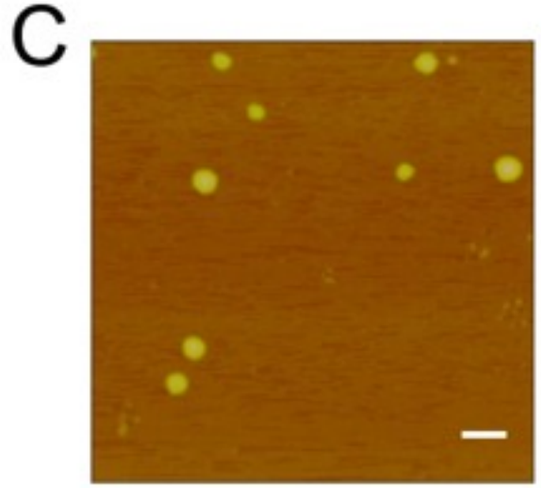
The modification-reversion process does not affect on the protein activity.

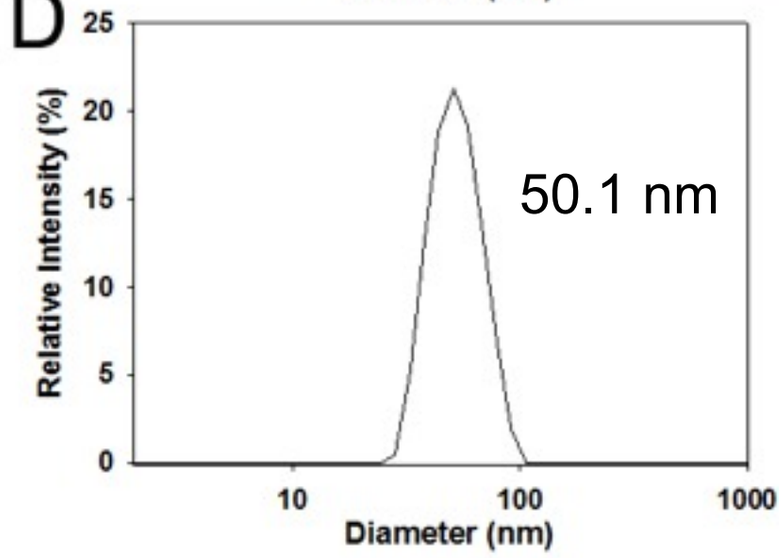
Formation of the PIC micelles at physiological salt condition





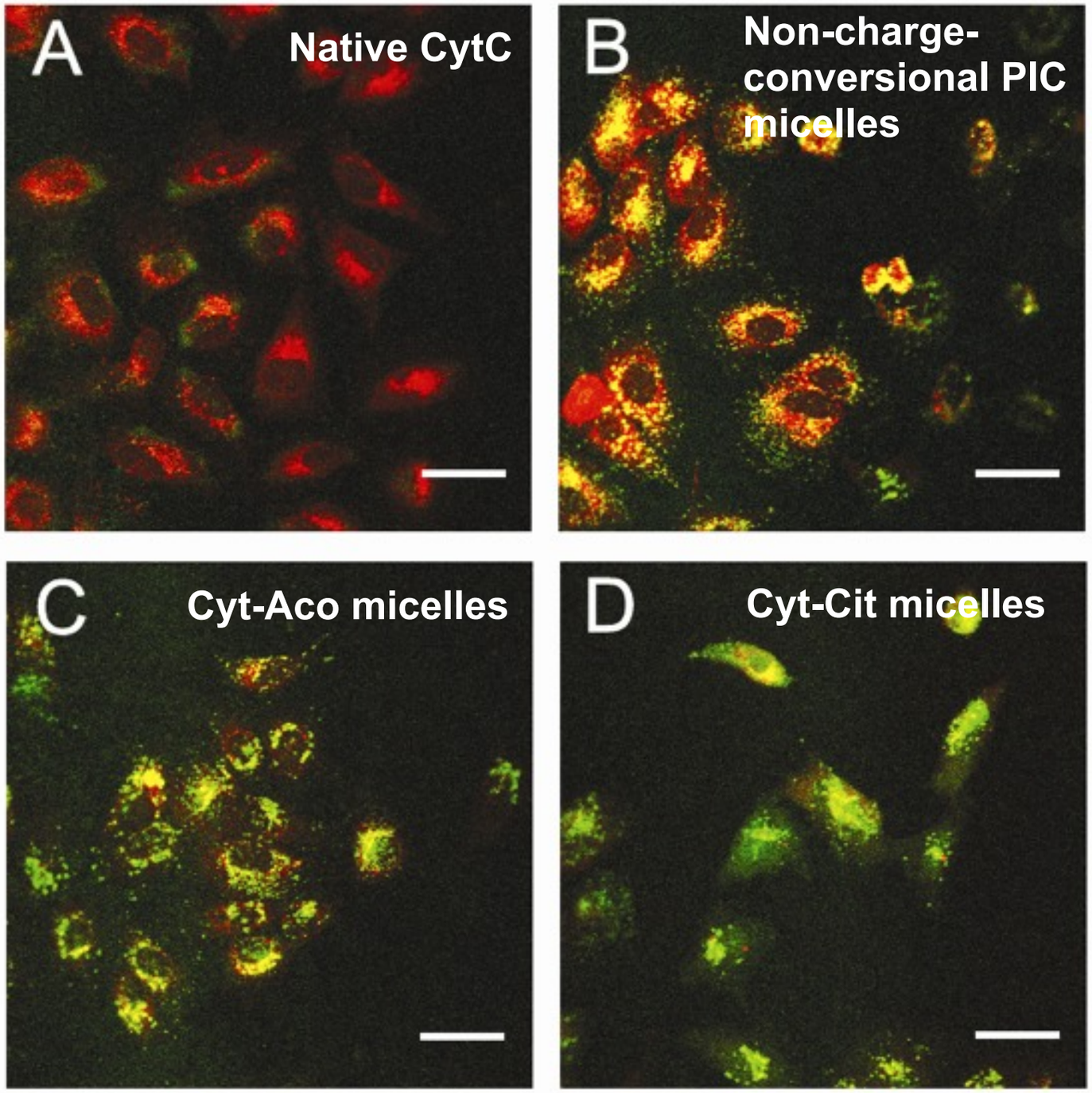






Stable micelles at 150mM NaCl!

Intracellular protein delivery by charge-conversional PIC micelles



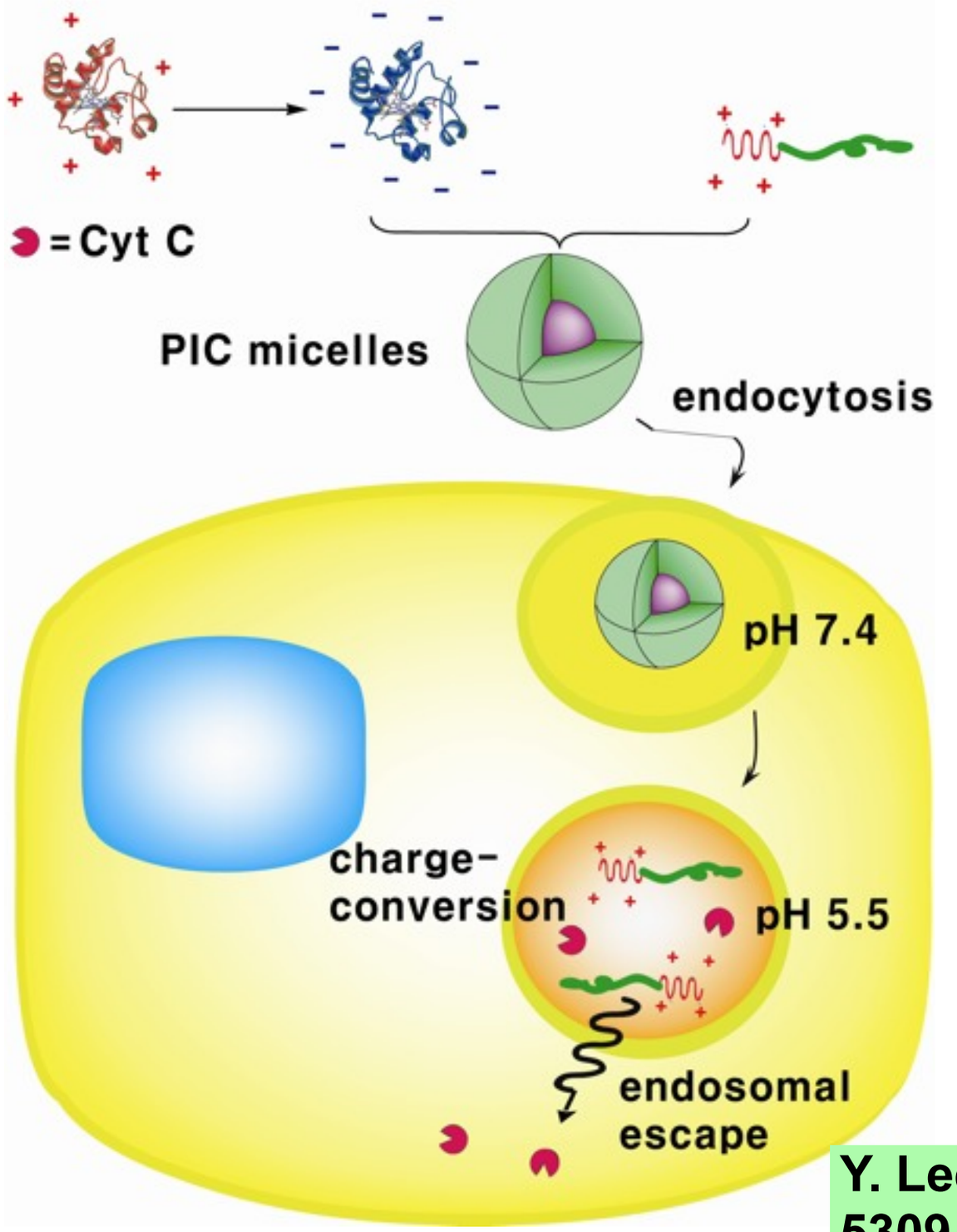
- Cyt-Alexa 488
  (protein escaped from endosome)
- Endosome-Lysotracker Red
  (endosome)
- Co-localization of green and red
  (Cyt in endosome)

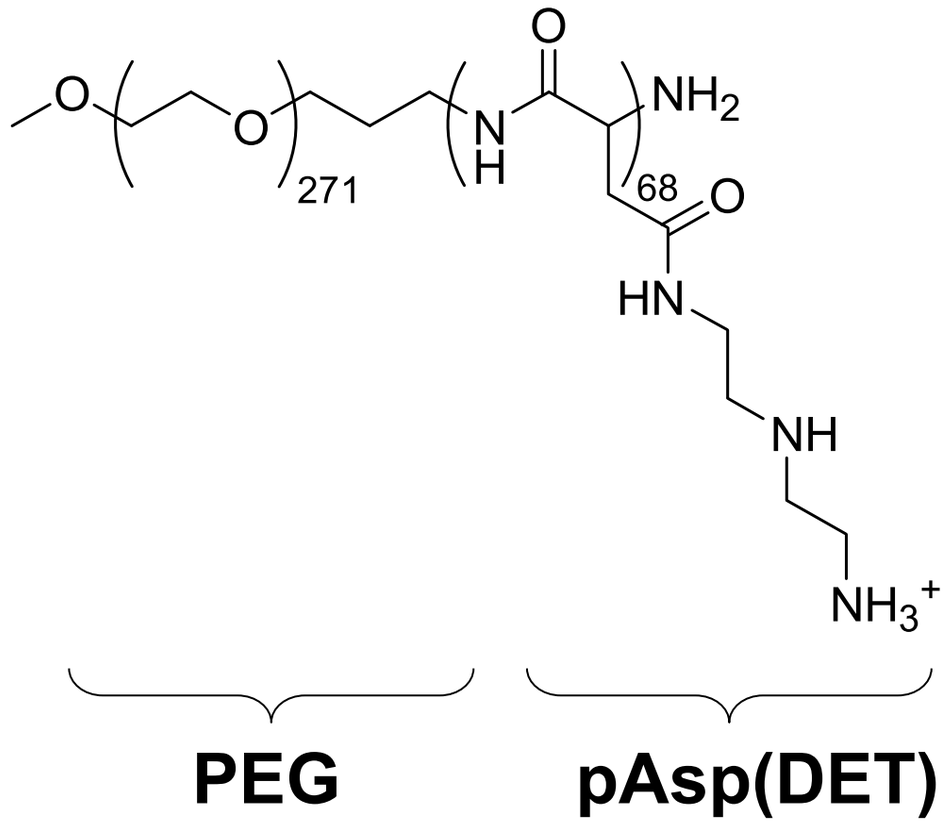
Cyt C: no internalization

Non-charge-conversional PIC micelles: efficient internalization (yellow) but no escape

Charge-conversional PIC micelles: efficient internalization and efficient escape (green)

Endosomal escape accelerated by PEG-pAsp(DET) polymer

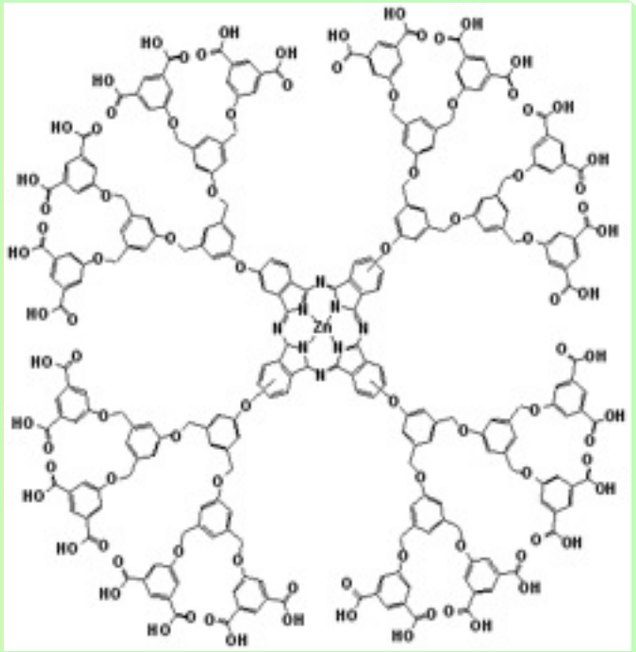




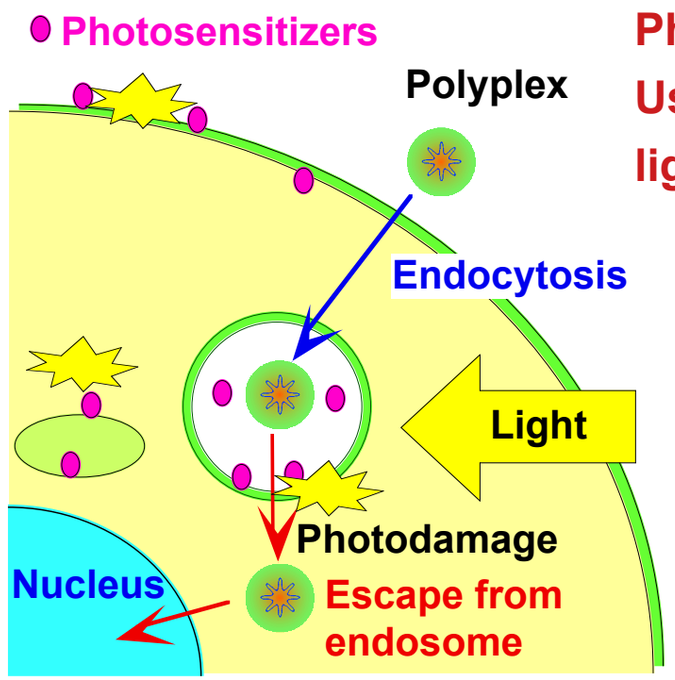
The released PEG-pAsp(DET) helps the endosomal escape.

Y. Lee, et al, *Angew. Chem. Int'l. Ed*. 48(29) 5309 (2009) (Highlighted as inside cover story)

Endosomal escape: A key issue in intracellular gene delivery



Challenge: Design of photosensitive polyplex with low cytotoxicity and high photochemical efficiency for site-directed gene transfer *in vivo*



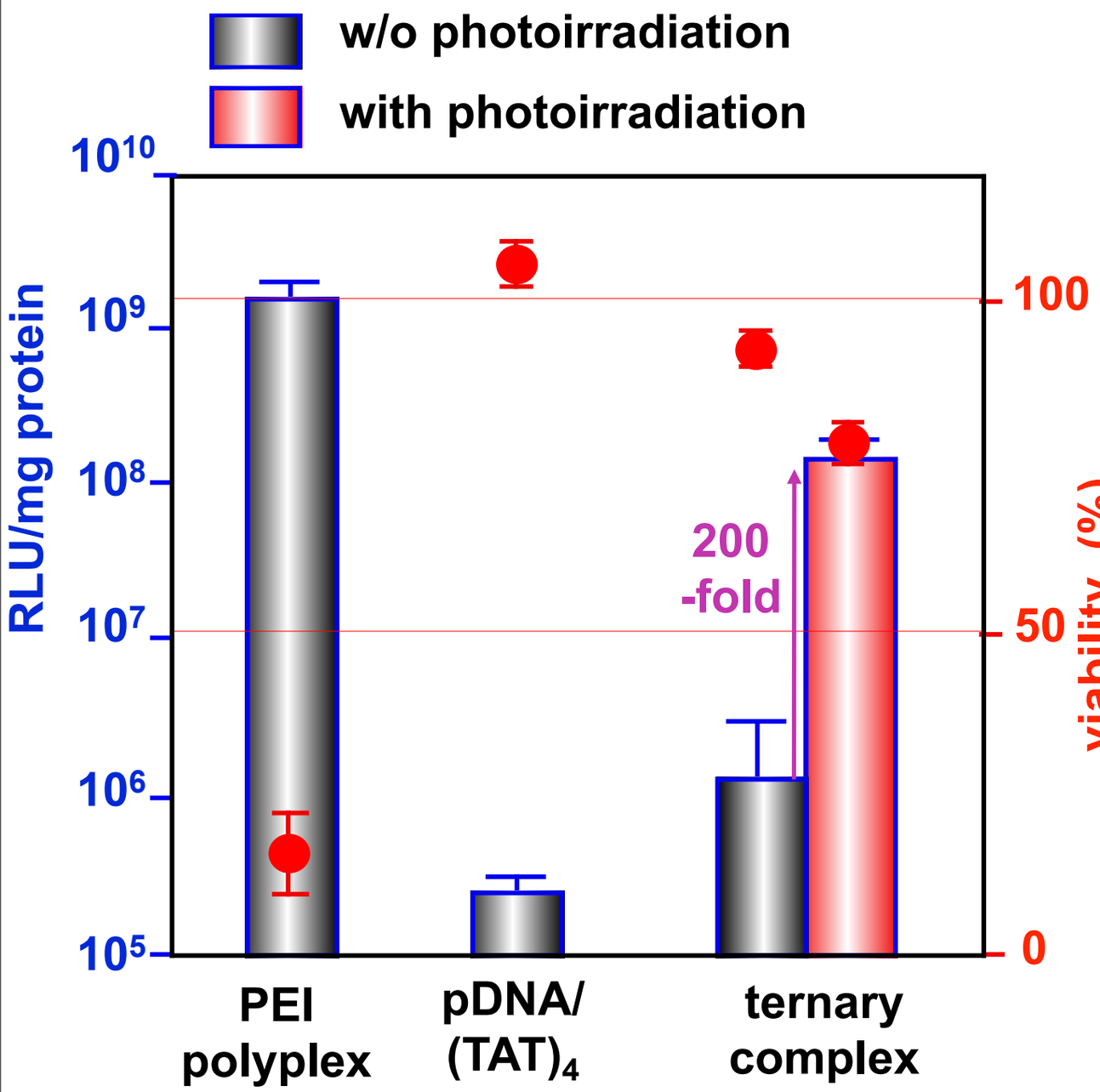
Photochemical transfection: Use of photosensitizer and light illumination

A. Hogest et al,
Hum. Gene Ther.
11, 869-880 (2000)

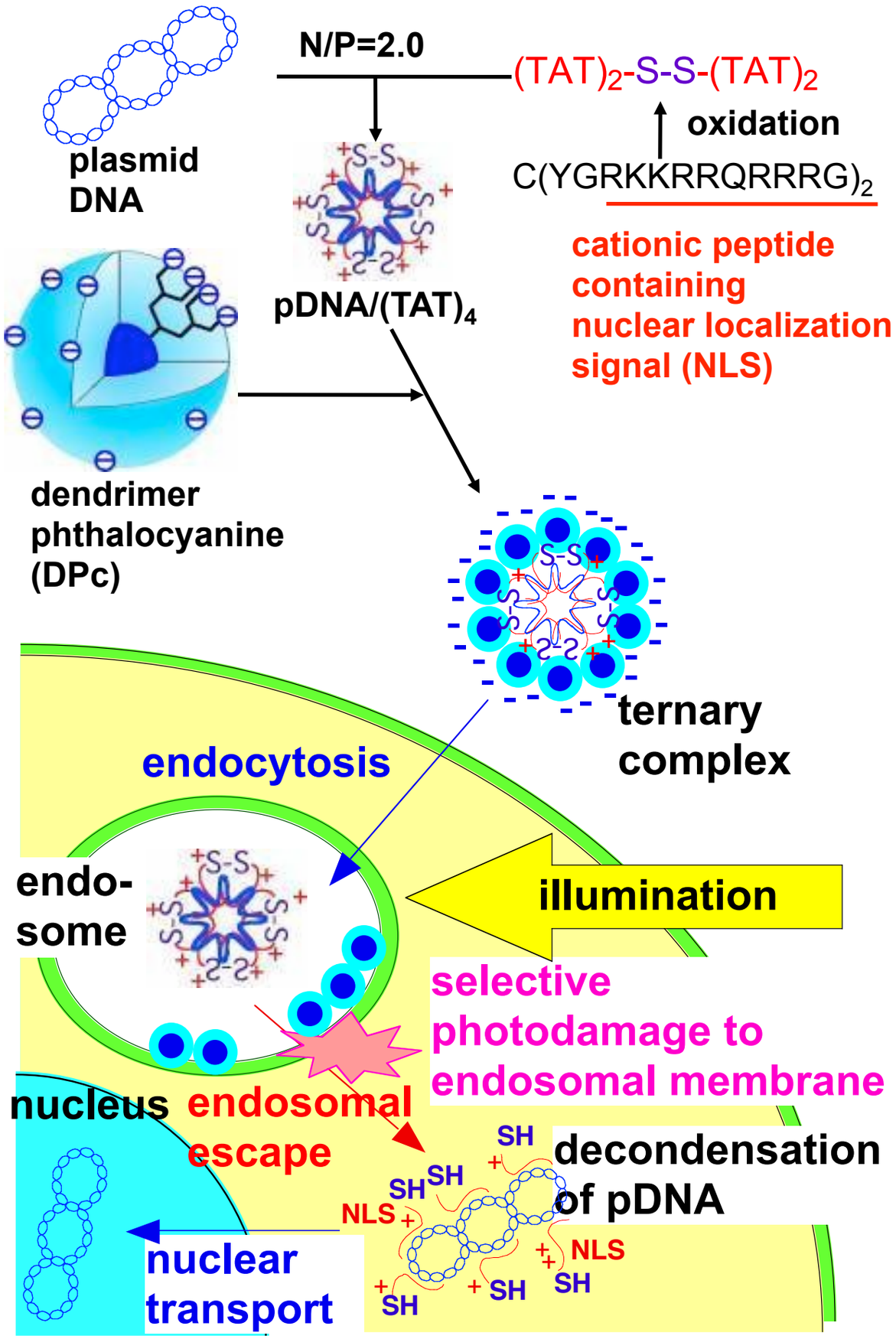
Temporal and spatial control of the transgene expression by light-responsive gene carriers

CNBI
Center for NanoBio Integration

Transfection efficiency and photocytotoxicity of the ternary complex (incubation time:48 h)



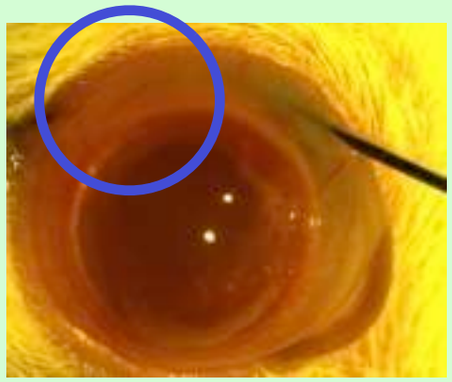
Remarkable photochemical enhancement of the gene expression was achieved with reduced photocytotoxicity



Intelligent gene carrier for temporal and spatial control of gene transfer in vivo

—Site-directed transfection using light-responsive gene carriers—

A part of conjunctiva in a rat eye was photoirradiated 2 h after subconjunctival injection of the ternary complex (150mL)



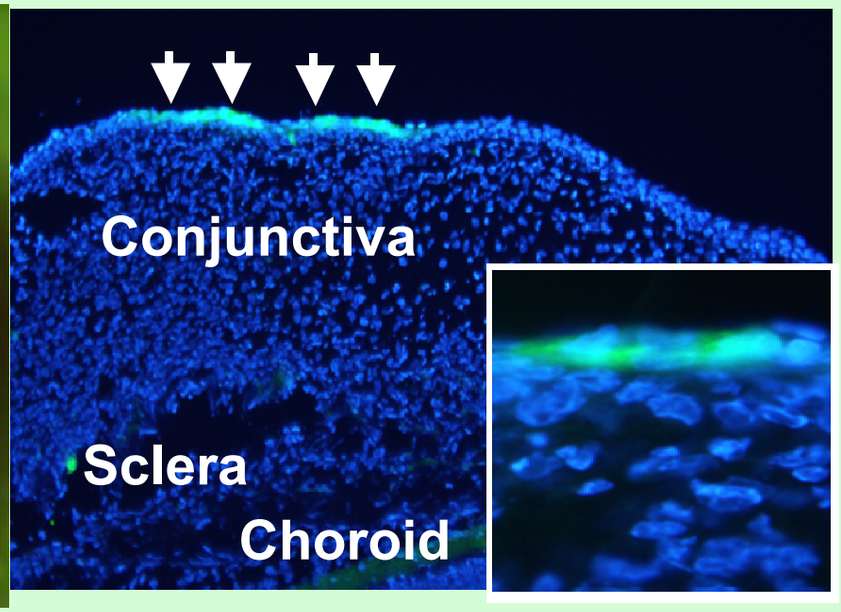
Light-induced, site-directed transfection of the YFP gene
(Collaboration with Dept. Ophthalmology, the University of Tokyo Hospital)

The fluorescent image of the YFP expression in a rat eye was observed by a stereoscopic microscope

Applications for gene therapy of ophthalmic diseases such as AMD

YFP expression only at the laser-irradiated site

laser irradiation



2 days after

N. Nishiyama, et al, *Nature Materials*, 4(12), 934-941 (2005)

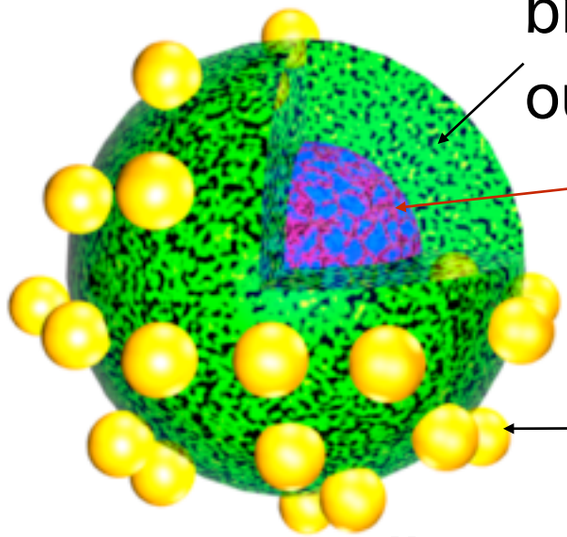
Supramolecular nanocarriers based on polyion complex formation

block copolymer

hydrophilic segment

charged segment

self -assembly



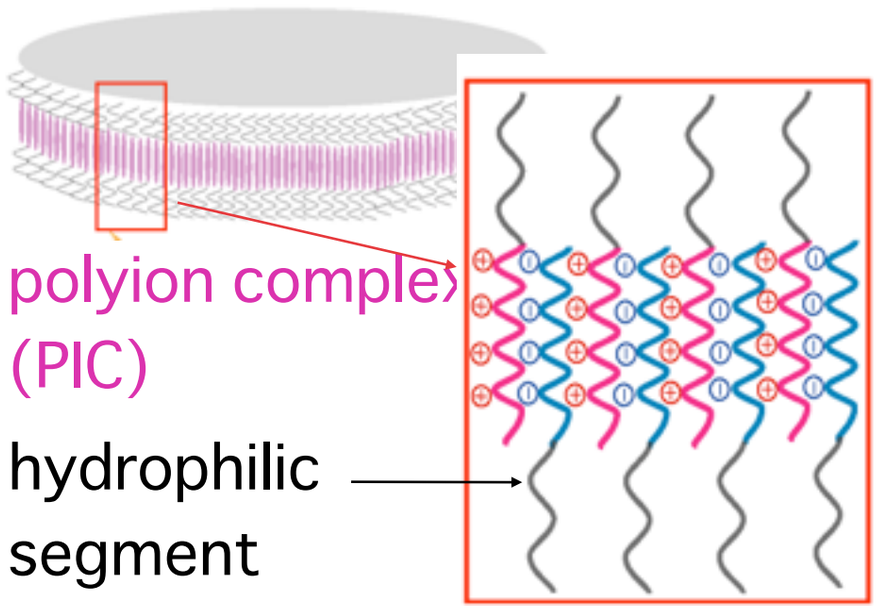
biocompatible outer shell

drug-loaded core

pilot molecule

PIC micelle

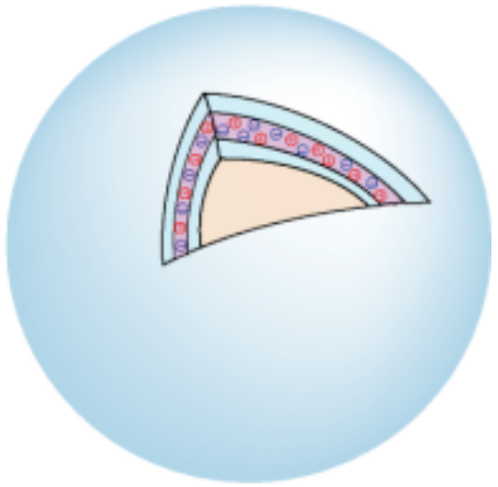
lamellar structure



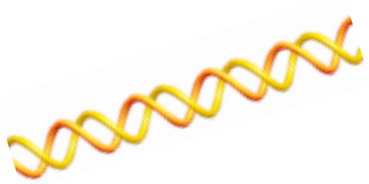
polyion complex (PIC)

hydrophilic segment

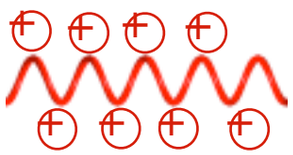
self -assembly



polymeric vesicular nanocarrier (PICsome)

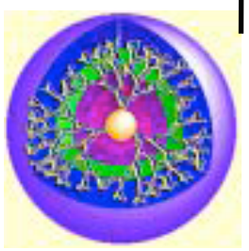


DNA

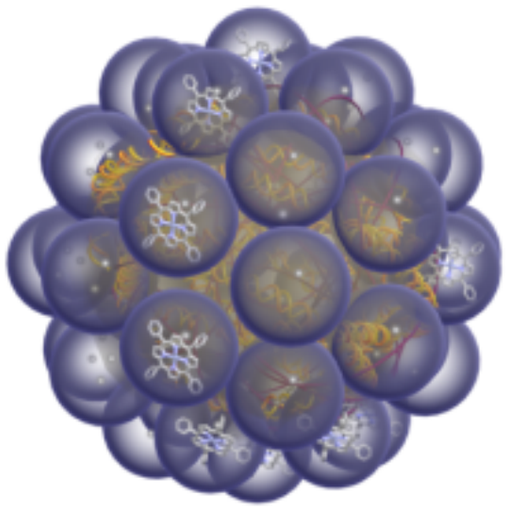


cationic polymer / peptide

self-assembly



anionic dendrimer photo-sensitizer



Polyplex enveloped with dendrimeric photosensitizer

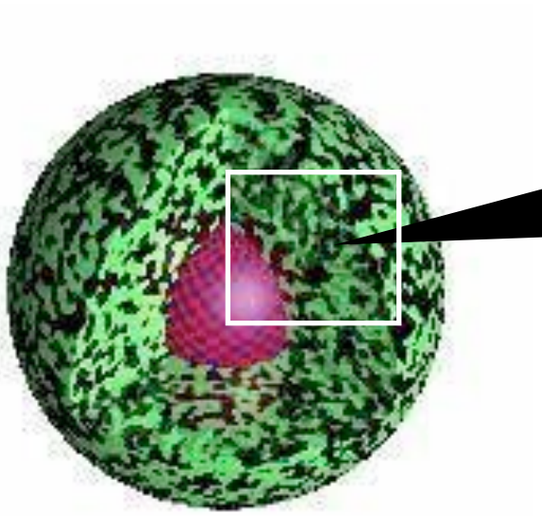
Molecular Strategies for PICsome Formation

* Stabilize lamella phase to prevent micelle formation

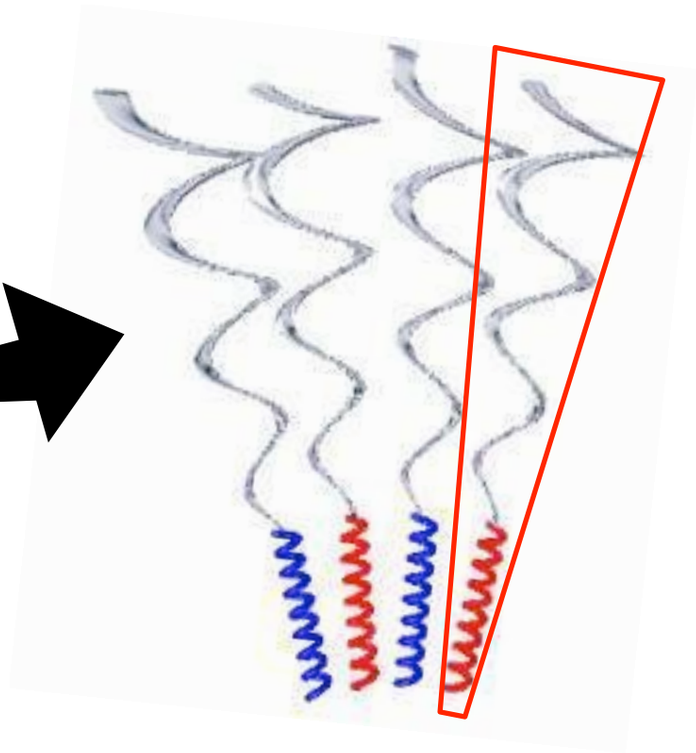
DP of PEG (DP_{PEG}) = 270

DP of Polyion Segments (DP_{PI}) = 70

→ **Formation of Micelles**



PIC Micelle



Curved Interface
between
PEG and PIC Layer

Lowered ratio of
DP_{PEG} to DP_{PI}

→ More Planar?

This Work

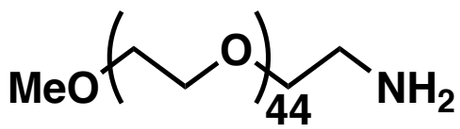
DP_{PEG} = 45

DP_{PI} = 100

Synthetic Scheme of Block Copolymers

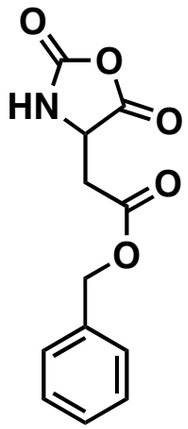
- Chain length matching of a pair of oppositely charged segments

PEG
(M_w = 2000)

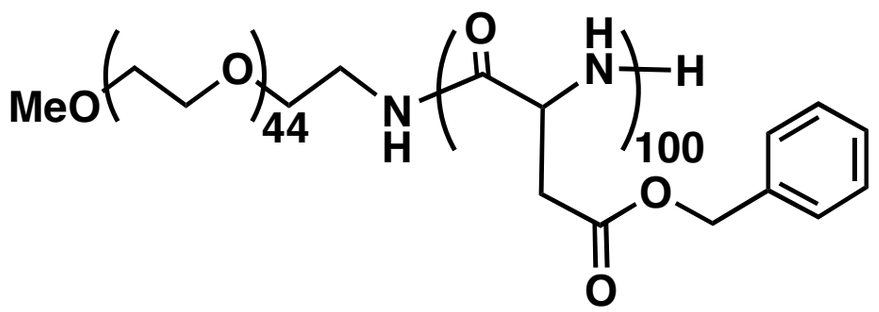


+ 100·

BLA–NCA



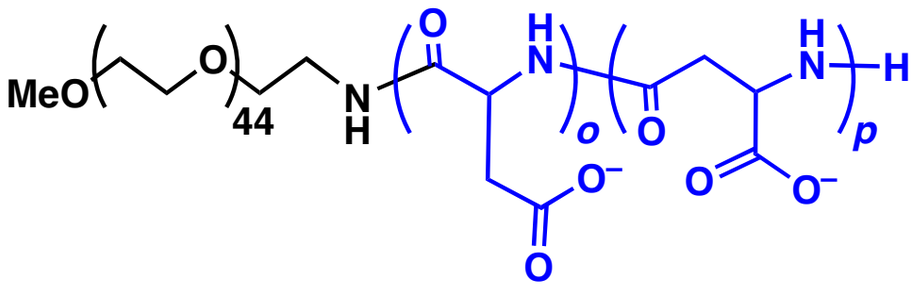
PEG–PBLA



PEG–PBLA

Hydrolysis

Polyanion (Polyaspartate)

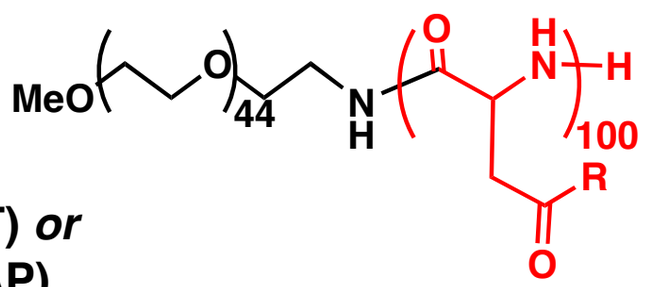


PEG–PAsp
(o + p = 100)

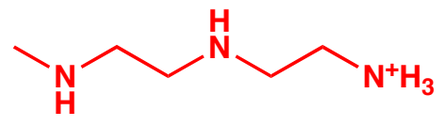
Aminolysis

diethylenetriammine (DET) *or*
1,5-diaminopentane (DAP)

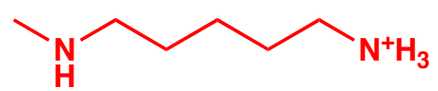
Polycations



R =

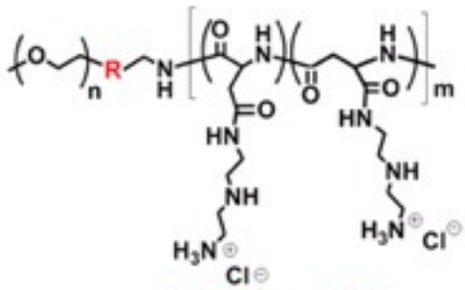


PEG–P(Asp–DET)

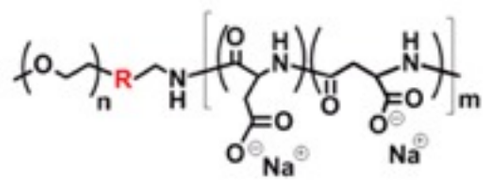


PEG–P(Asp–DAP)

"Self-templating" strategy for hollow nanocapsule preparation



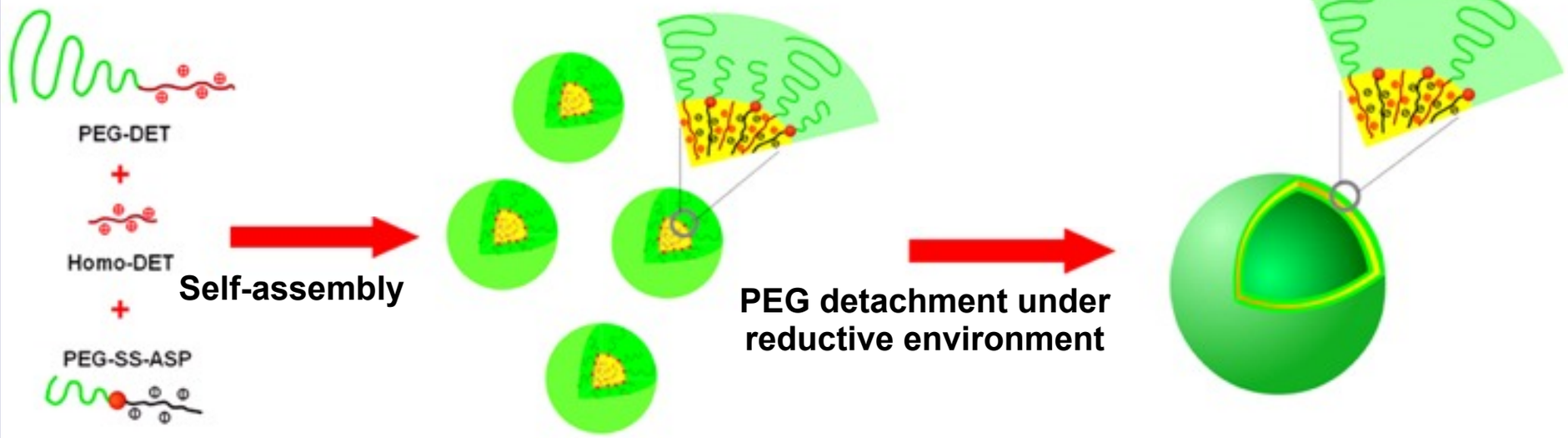
R = CH_2
PEG-PAsp(DET) 12K-68

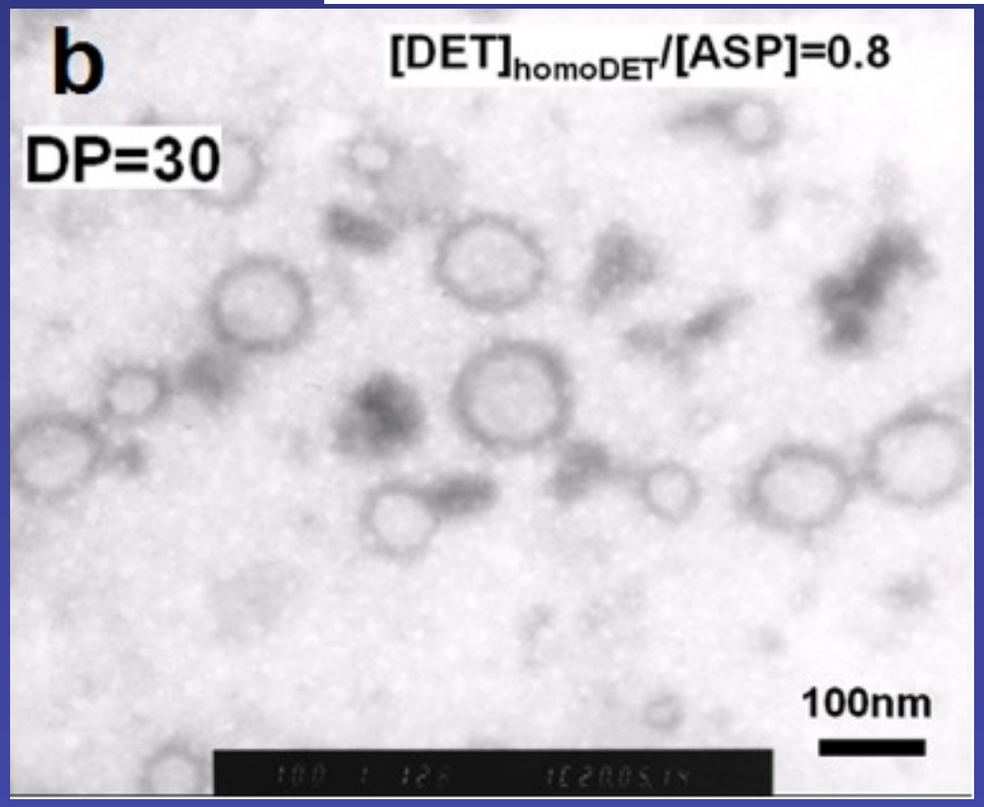


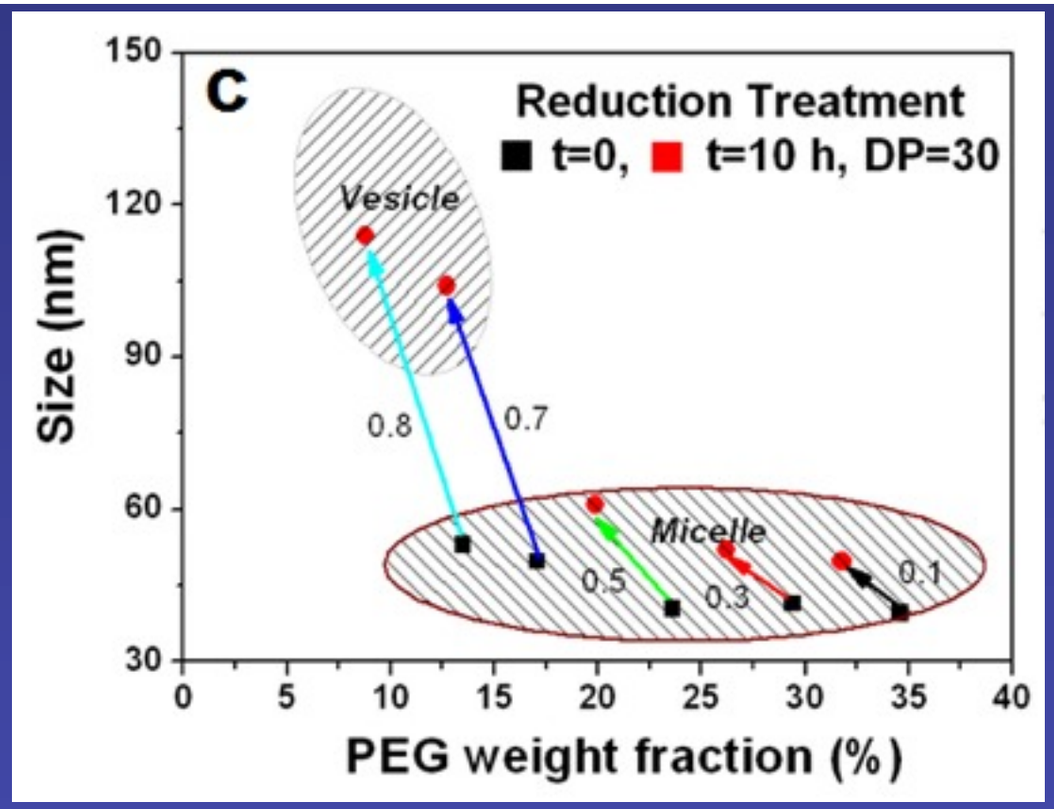
R = SS
PEG-SS-PAsp 2K-91

PEG chains

Polyion complex cores



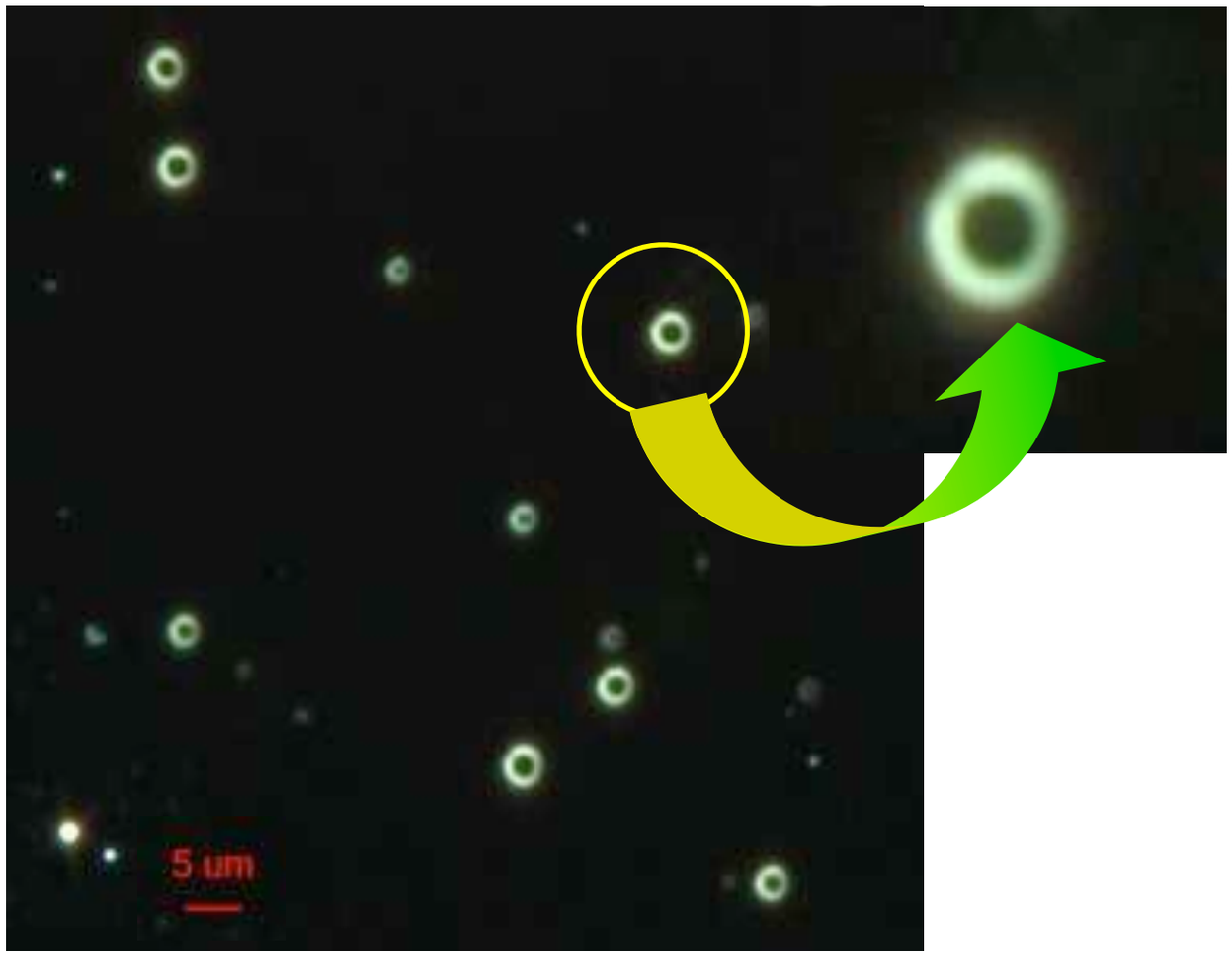




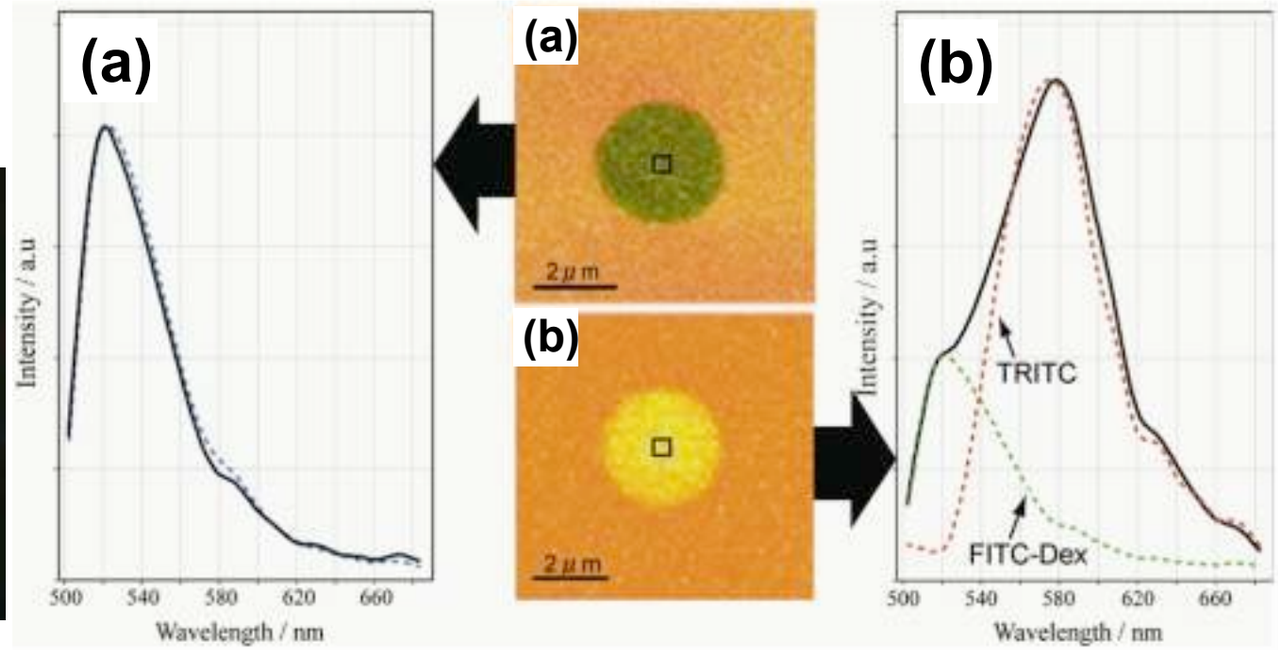
PEG weight fraction is a crucial factor for micelle-to-vesicle transition

Dong, W. F. et al, JACS, 20,1664,2009.

Observation of Hollow Structure of PICsome



Dark-field microscopic image of PICsome



(a) FITC-encapsulated PICsome + TRITC-dextran (Mn=65,000~76,000)

(b) FITC-encapsulated PICsome + TRITC (Mw=443)

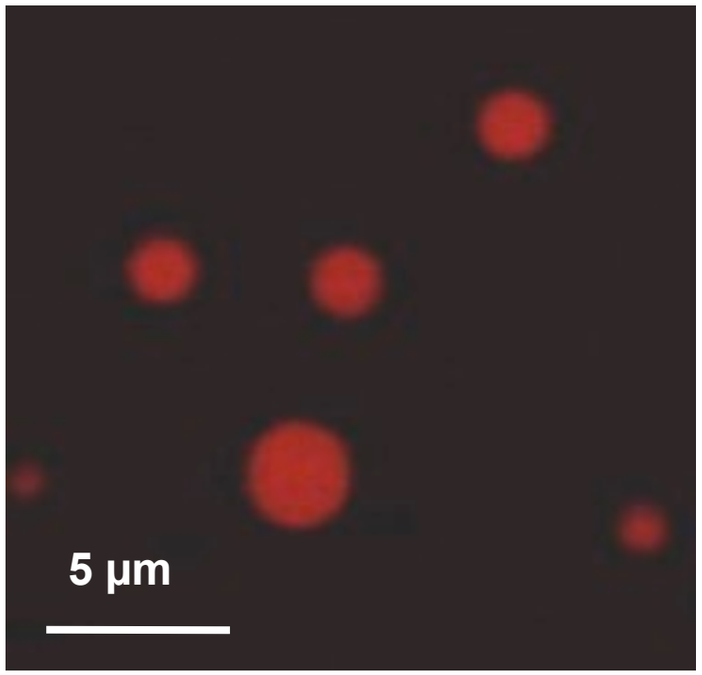
Semi-permeable nature of PIC membrane

Confocal laser scanning microscopic image of PICsome with entrapped FITC-dextran

A. Koide, et al, *J. Amer. Chem. Soc.*, 128, 5988-5989 (2006)

Myoglobin-encapuslated PICsome as Nanobio-reactor

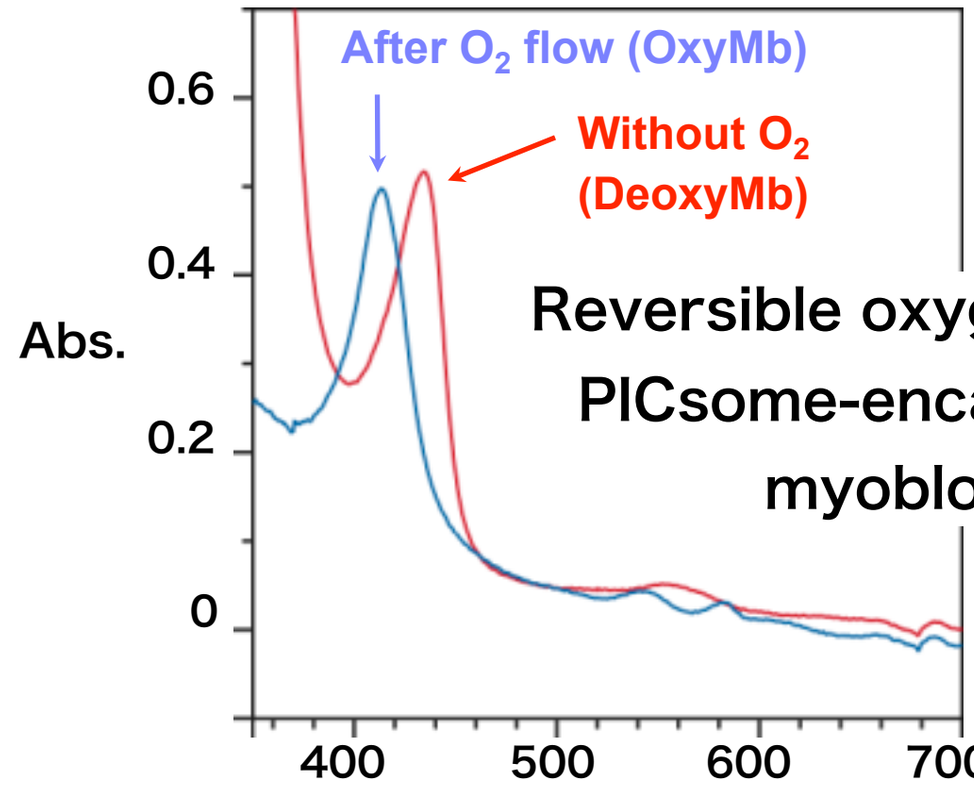
Encapsulation of oxygen carrier protein (myoglobin) (Mw ＝17,800)



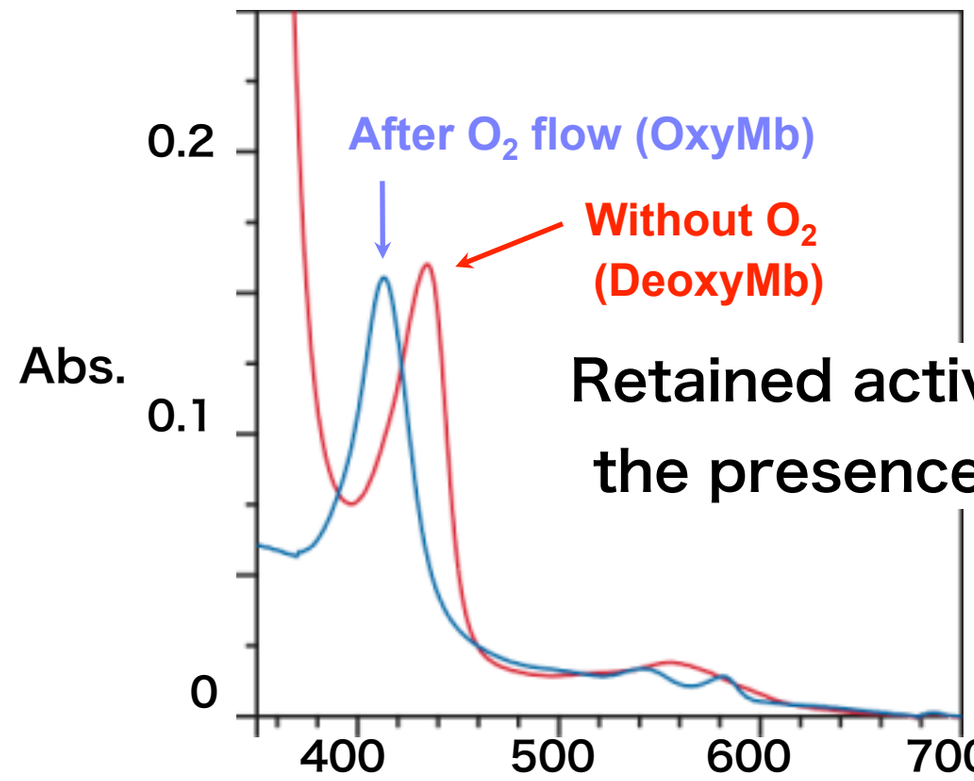
CLSM image of rhodamin-labeled myoglobin in PICsome

Activity evaluation

Trypsin addition



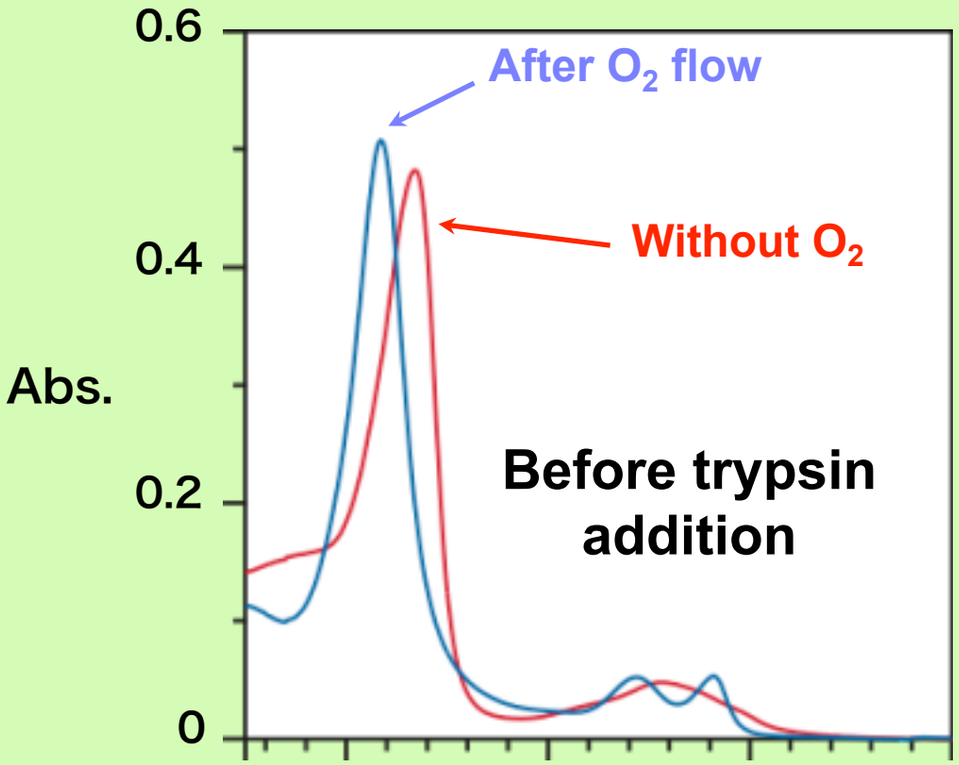
Reversible oxygenation of PICsome-encapsulated myoblobin

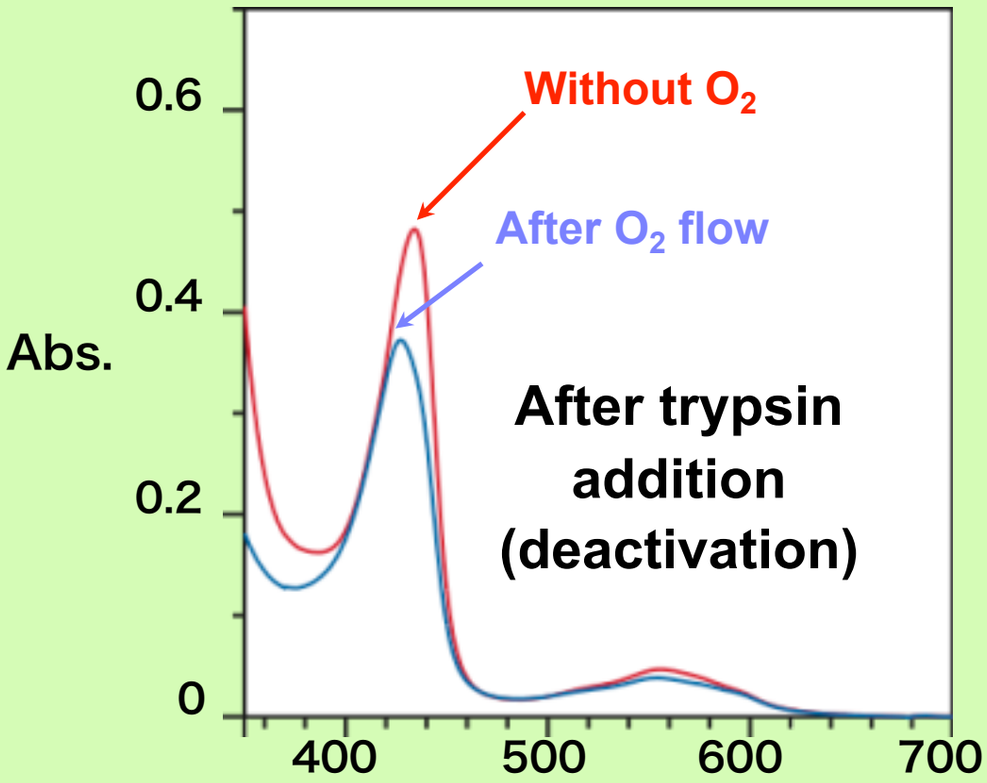


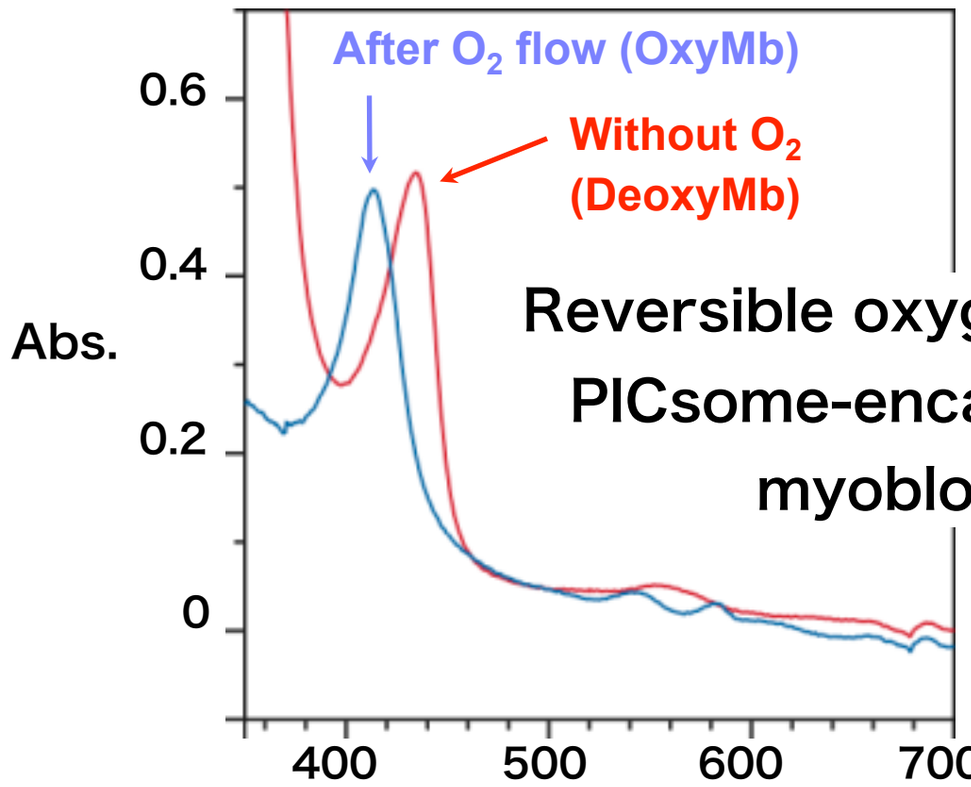
Retained activity even in the presence of trypsin

Myoglobin-encapuslated PICsome as Nanobio-reactor

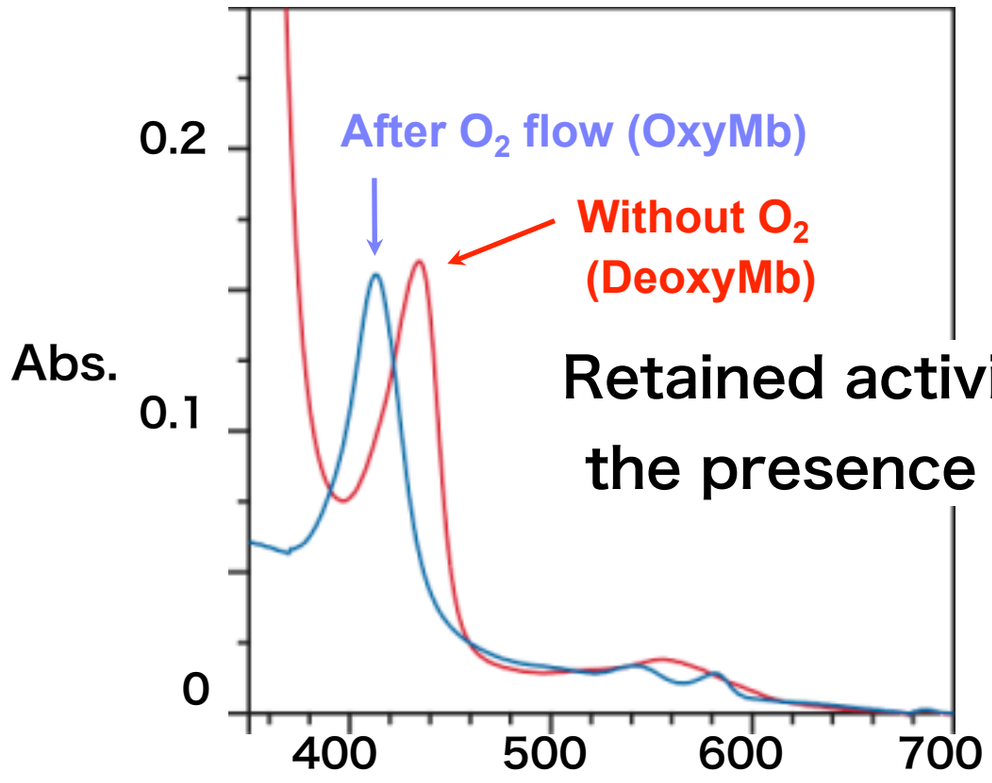
Free myoglobin





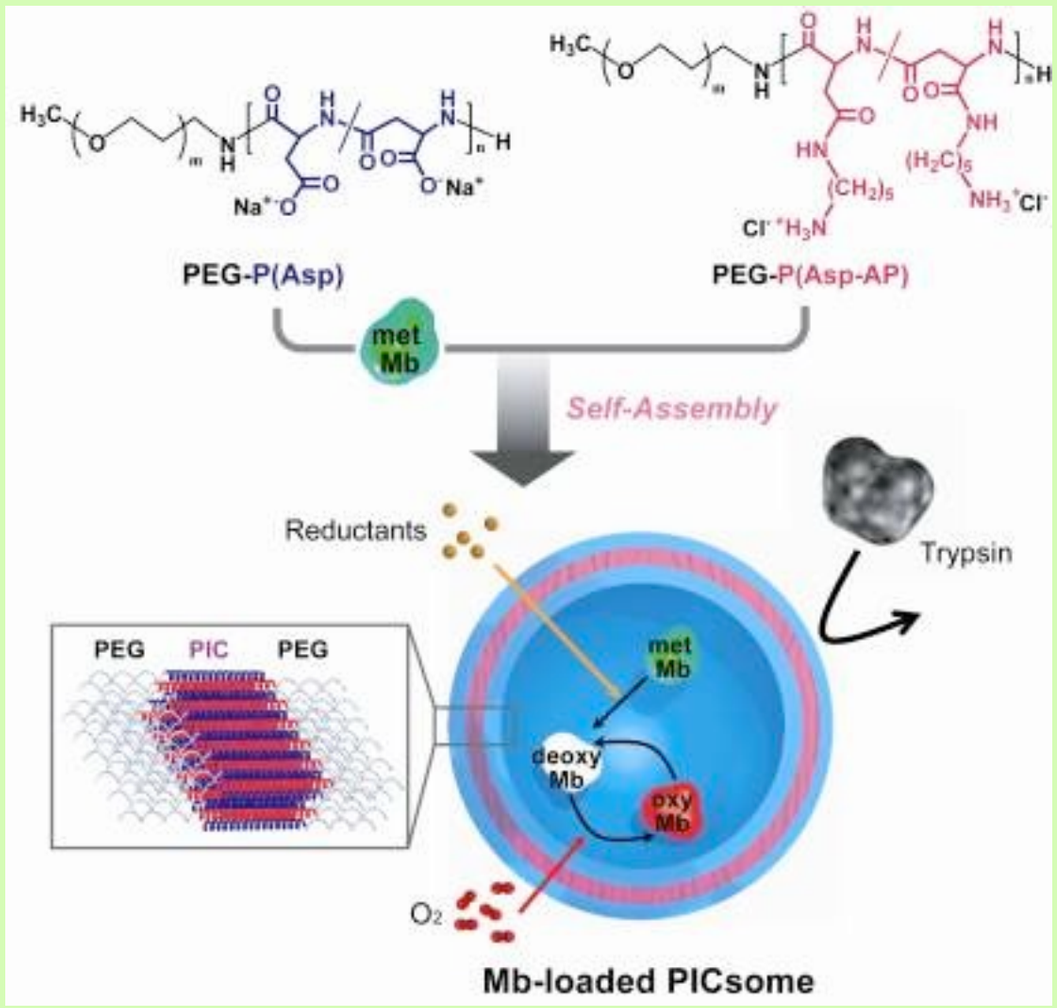


Reversible oxygenation of PICsome-encapsulated myoblobin



Retained activity even in the presence of trypsin

Myoglobin-encapuslated PICsome as Nanobio-reactor

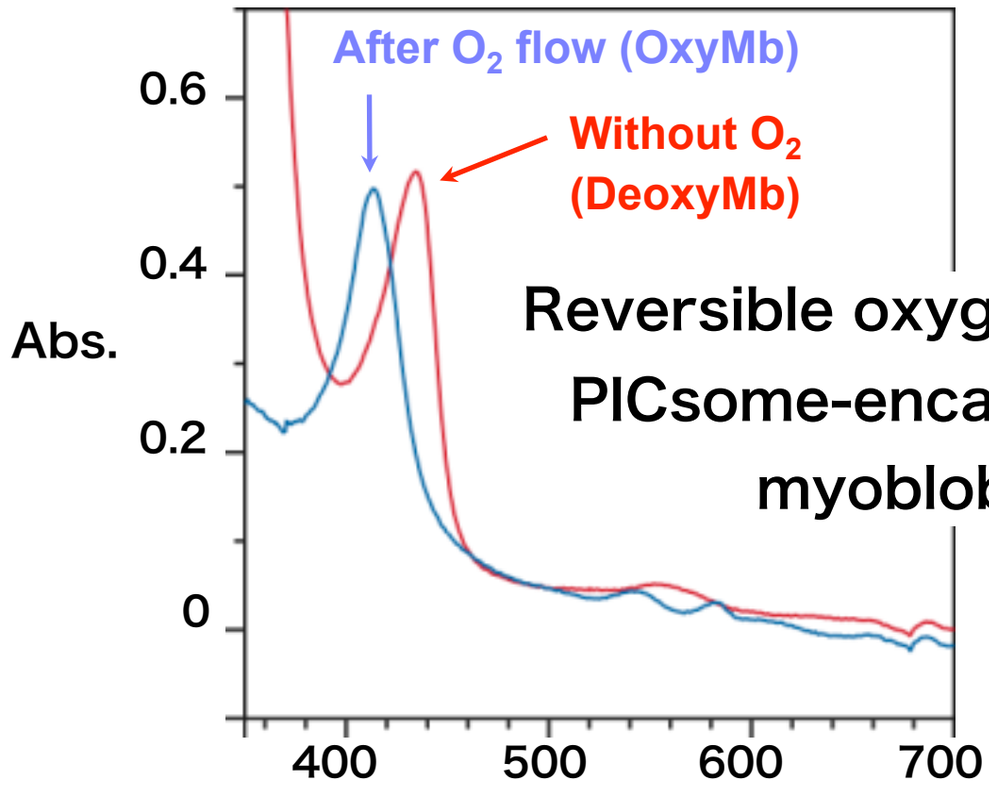


*Reversible O_2-binding

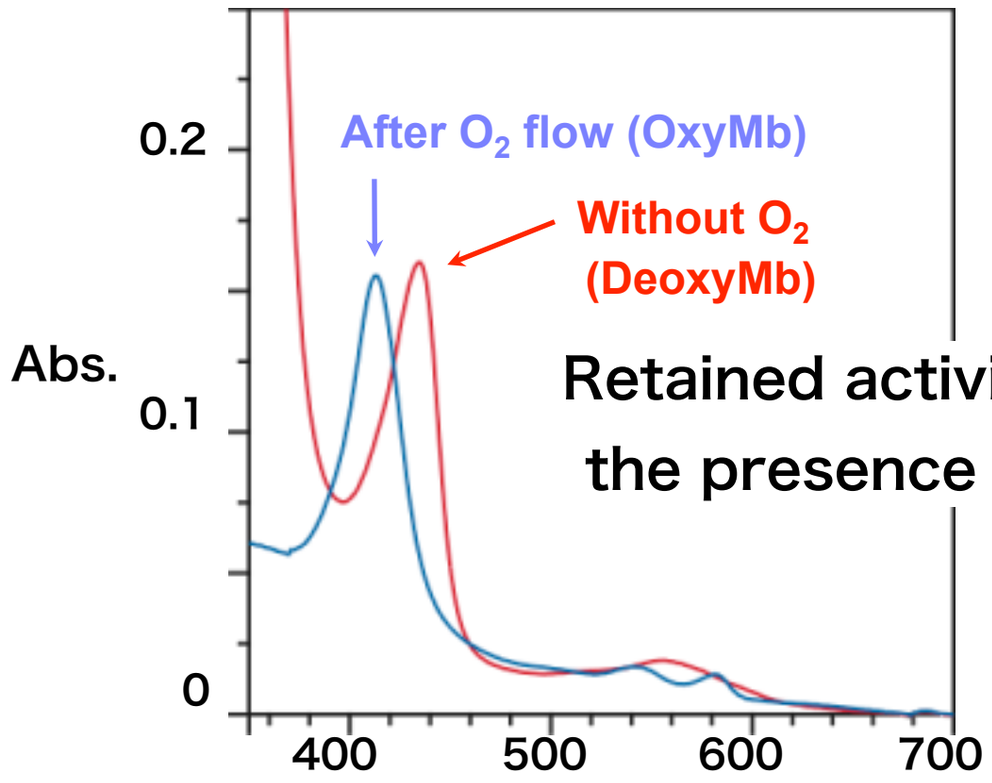
*High Proteinase Resistance

→ Promising Carrier for Protein Delivery

A. Kishimura, et al,
Angew. Chem. Int'l. Ed., 46, 6085-6088 (2007)

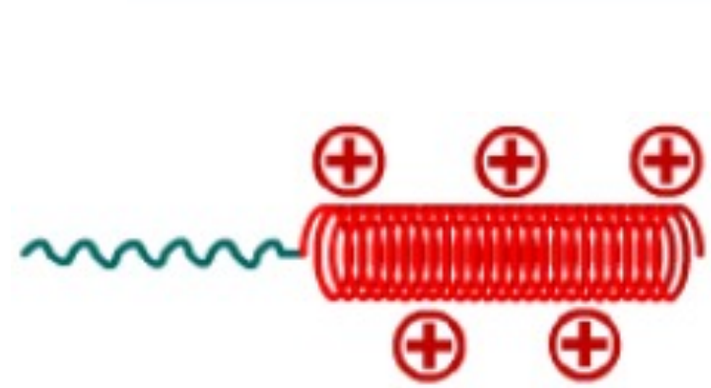


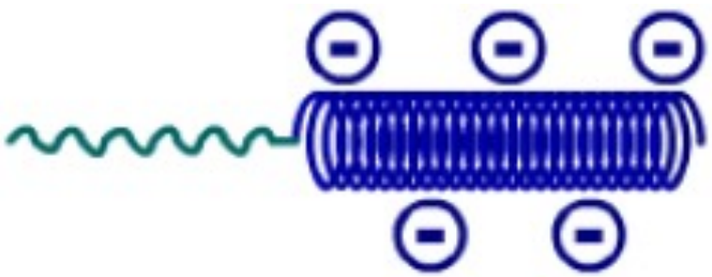
Reversible oxygenation of PICsome-encapsulated myoblobin



Retained activity even in the presence of trypsin

PICsome as Functional Nano-container



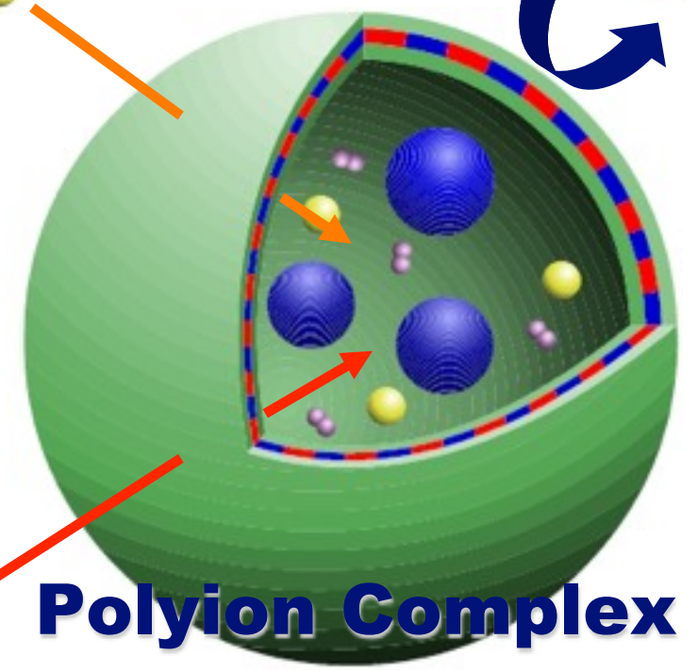


Cargo molecule

Prepare just by Mixing!!

Small Molecules

Enzyme



Polyion Complex Vesicle

PICsome

Oxygen

Utilities

- Encapsulating Macromolecules
- Selective Permeability
- Proteinase Resistance
- pH Sensitivity

Applications

- ★ Bio-reacter
- ★ Artificial Organelle
- ★ Artificial Cell
- ★ **Delivery Carrier**

Koide, A. et. al. ***J. Am. Chem. Soc****. 2006, 128, 5988., Kishimura, A. et. al.* ***Angew. Chem. Int. Ed.*** *2007, 46, 6085., Kishimura, A. et al.* ***Soft Matter****, in press*