

A bi-functional block copolymer hydrogels and micelles for protein and drug delivery

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● Stimuli for Sensitive Polymers

Minute change in
stimuli

Temperature

Electric Field

Light

Stress

pH

Ion species

Ionic strength

Solvent

composition

Biomolecules



Large change
in response
(Transition)

Conformation

Solubility

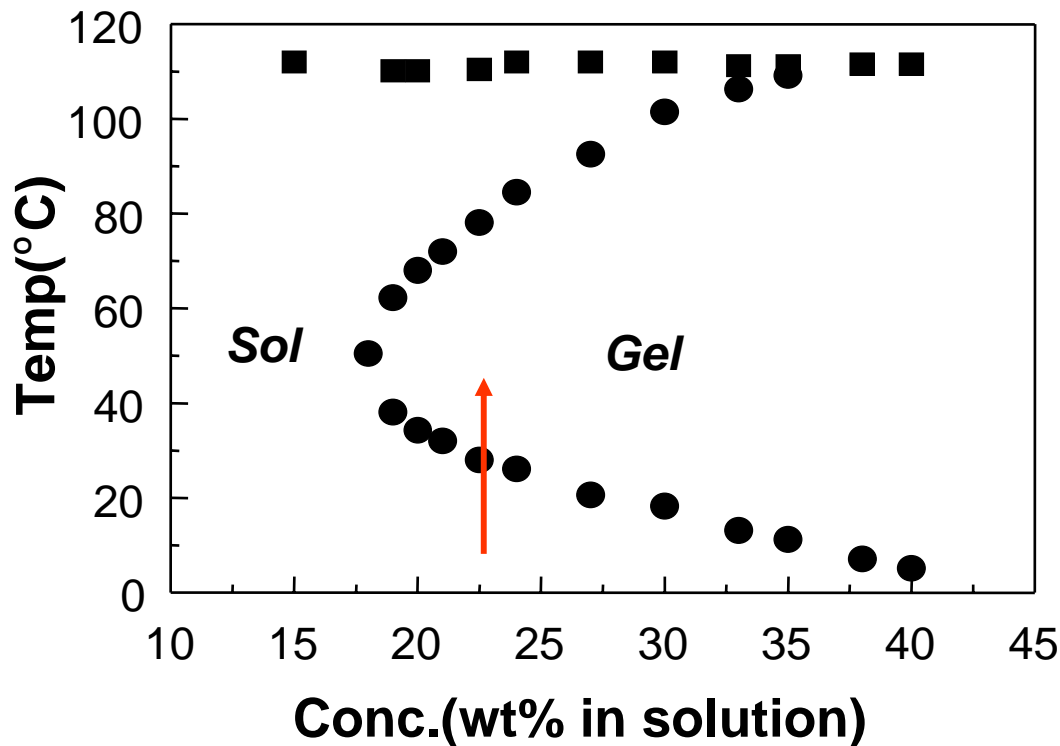
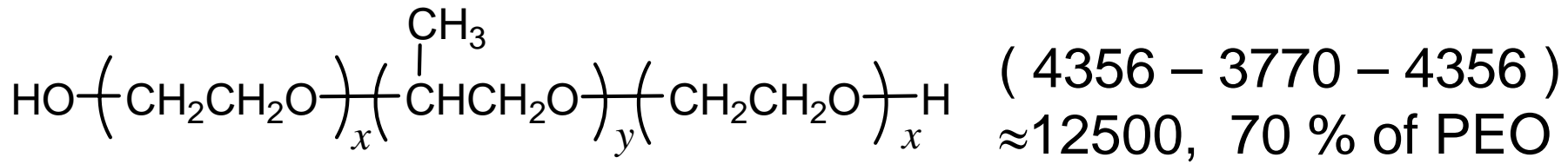
Shape

Swelling

Permeation

Temperature Sensitive Block Copolymer Hydrogel

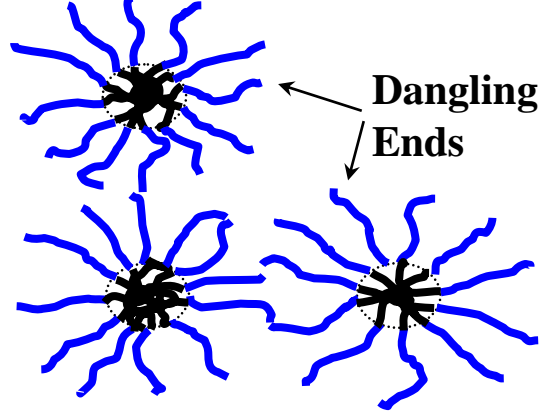
● Pluronic F-127 (PEO₉₉-PPO₆₅-PEO₉₉)



- Soluble in water
- Thermosensitive hydrogel
- **Concentration dependent**
- **Nonbiodegradable**

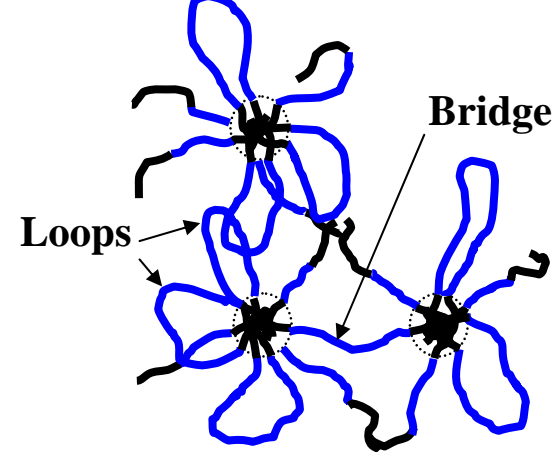
• Possible Micellar Chain Topologies in Water

PEO-PPO-PEO



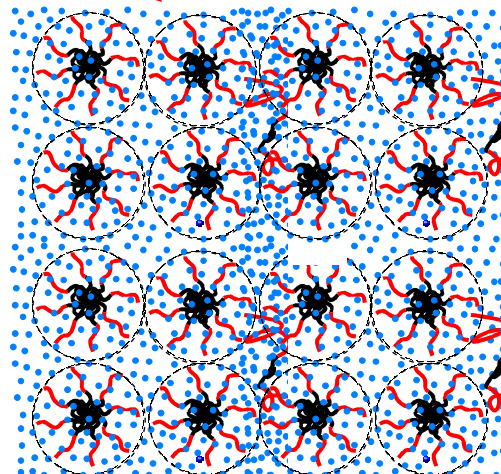
Diblock and A-B-A triblock copolymer micelles

PLGA-PEO-PLGA



B-A-B triblock copolymer micelles

Change temperature



Gelation by
micelle packing
and bridging

- **Biodegradable Thermosensitive Hydrogels**



Pluronic F-127 : PEO ---- PPO ---- PEO

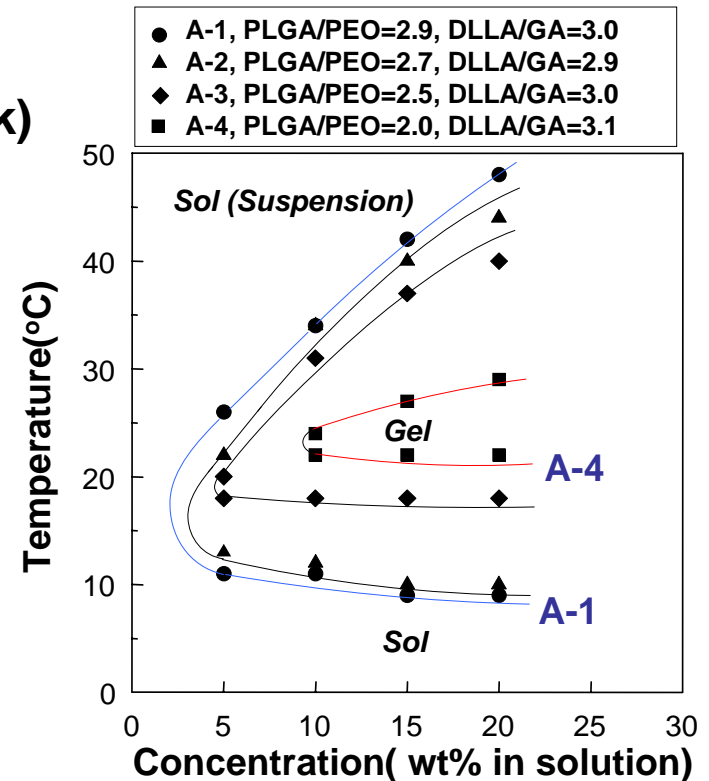
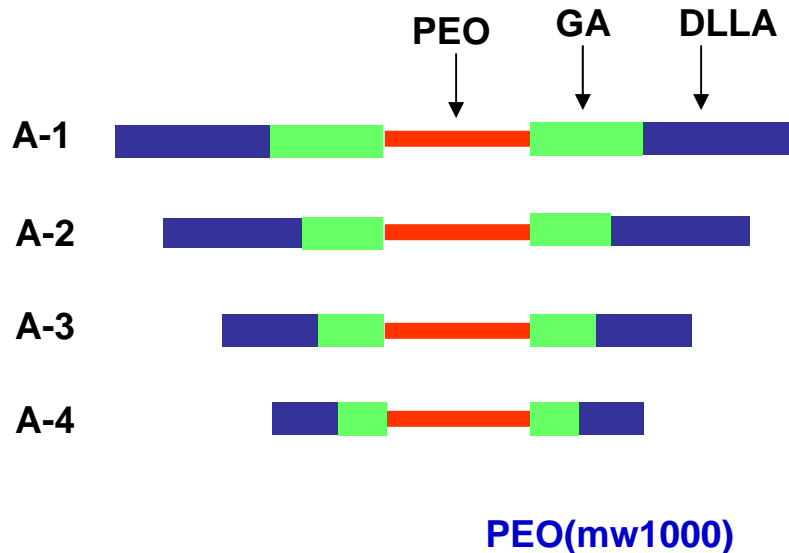
PPO \longleftrightarrow **Biodegradable Polymer**
PLA, PGA, PCL, PLGA, PCLA, etc

ABA type : PEO ---- PLGA ---- PEO

BAB type : PLGA ---- PEO ---- PLGA

● Control of Phase Diagram of **PLGA-PEO-PLGA**

- Different PLGA/PEO weight ratio
- Same DLLA/GA ratio in PLGA (random block)



pH and Temperature Sensitive Block Copolymer Hydrogels

• pH and Temperature Sensitive Block Copolymer Hydrogels



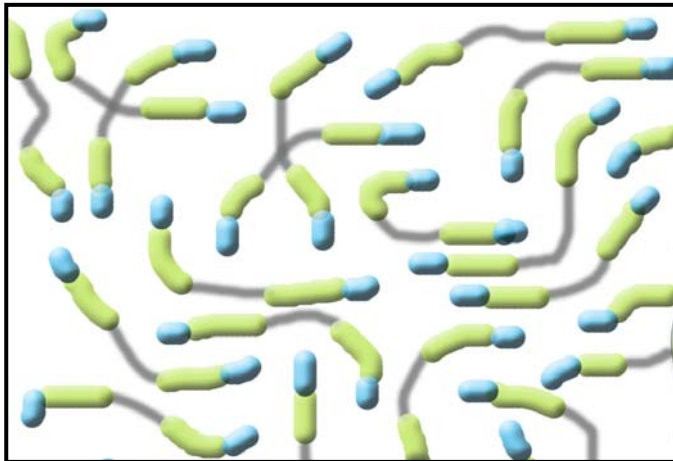
temperature sensitive blocks



hydrophobic blocks

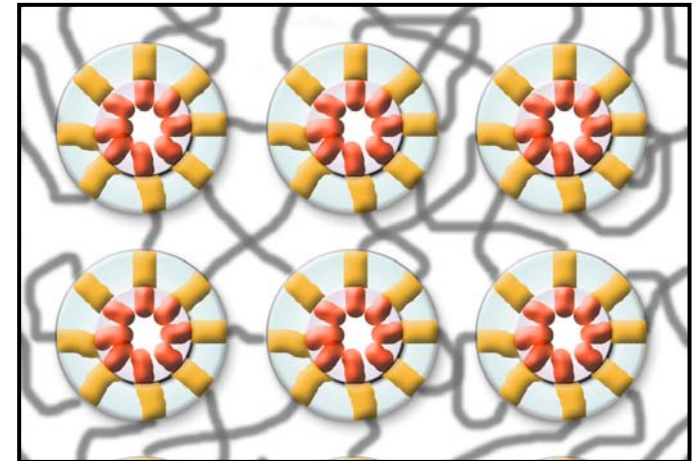
pH : pH sensitive block

PCL : polycaprolactone,
biodegradable polymers



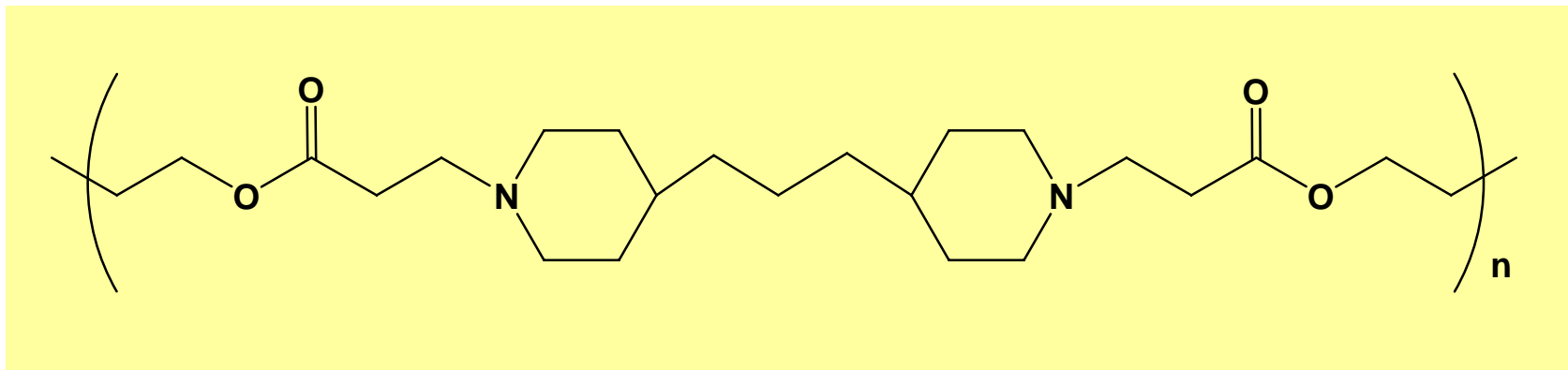
Sol

pH & temperature
change



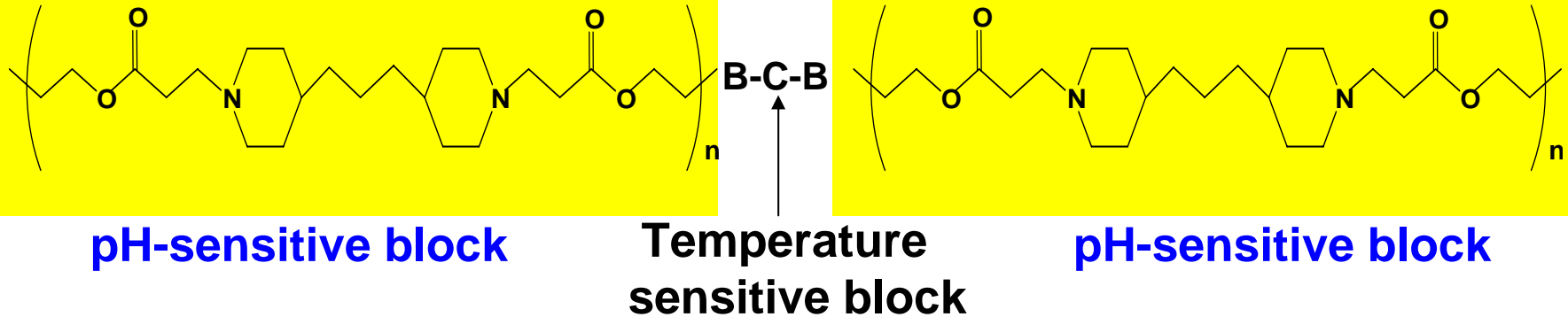
Gel

● Basic pH-sensitive Moiety – Poly(β - amino ester)



- Poly(β amino ester) was reported as a **pH-responsive biodegradable polymer**
- This polymer becomes **rapidly soluble at a pH below 6.5**
- The degradation of poly(β amino ester) occurred more slowly at pH 7.4 than at pH 5.1, while other polymers generally degraded more slowly at pH 5.1 than at pH 7.4
- At physiological pH, this polymer **interacted electro-statically with plasmid DNA** and formed nanometer-scale polymer/DNA complexes
- Poly(β amino ester) is **non-cytotoxic** and they degrade into nontoxic small molecule by products

• Ionization / deionization of Basic Moiety



Basic moiety

$pK_b (< 7.4)$

ionization

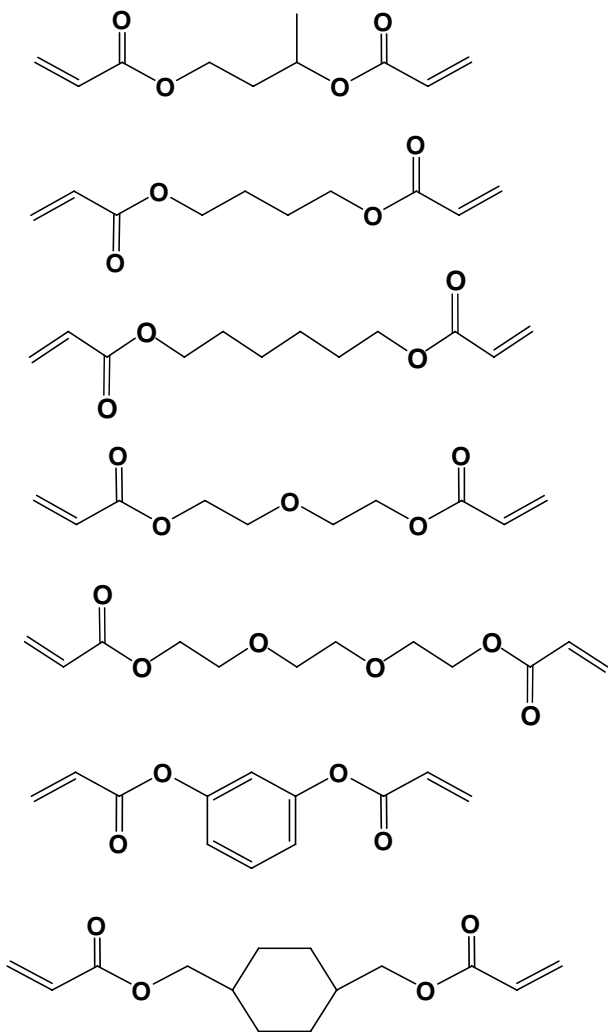
deionization

sol

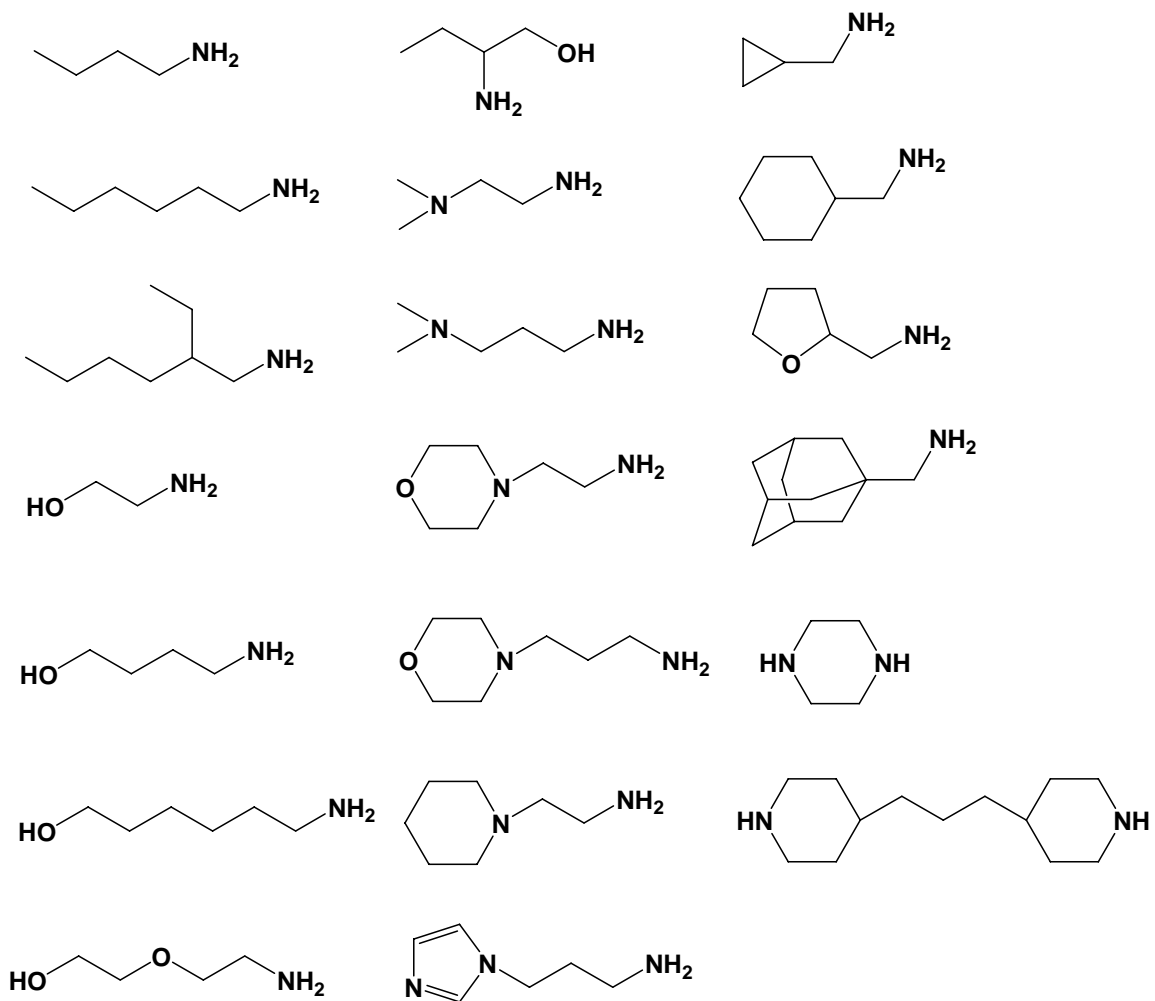
gel

● Monomers of Poly(β - amino ester)

Diacrylate

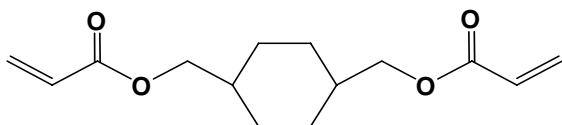
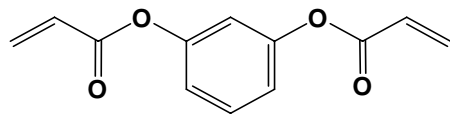
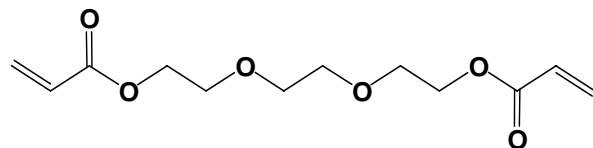
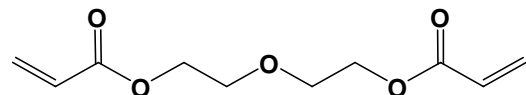
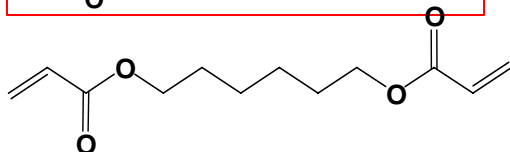
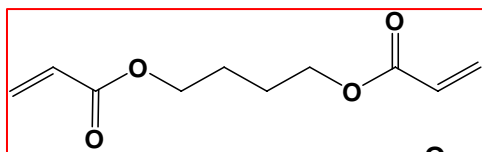
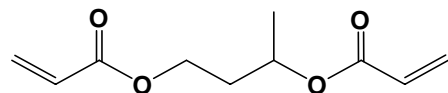


Amine

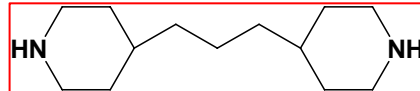
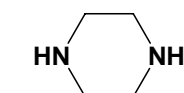
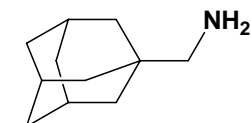
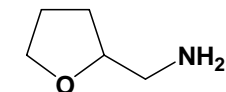
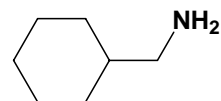
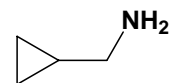
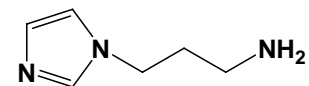
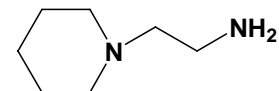
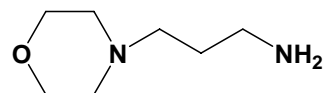
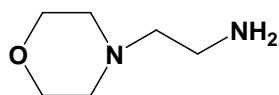
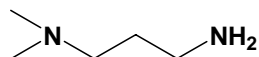
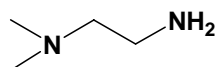
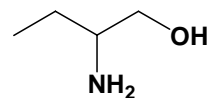
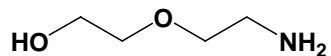
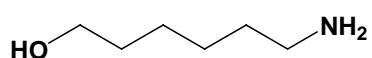
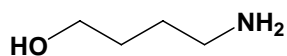
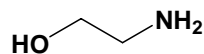
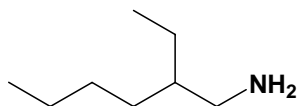
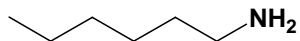
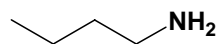


● Monomers of Poly(β - amino ester)

Diacrylate

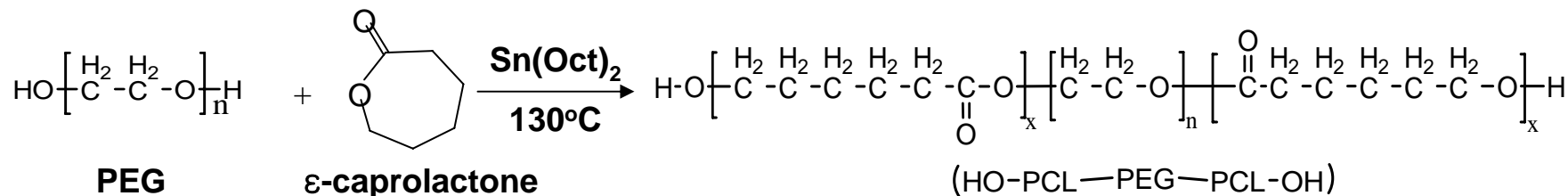


Amine

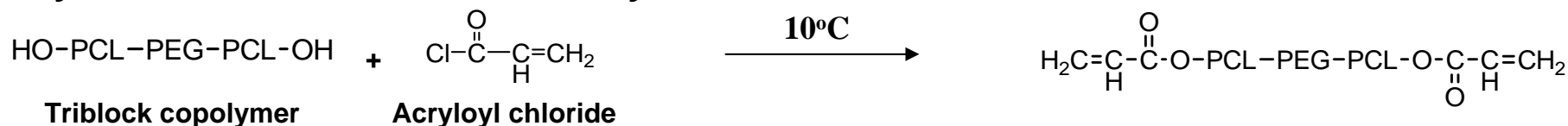


• Synthesis of PAE-PCL-PEG-PCL-PAE

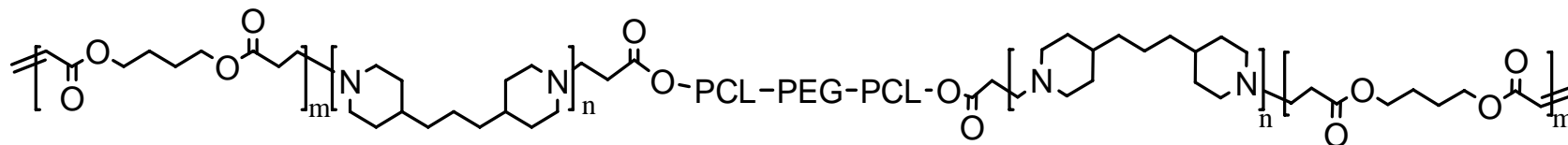
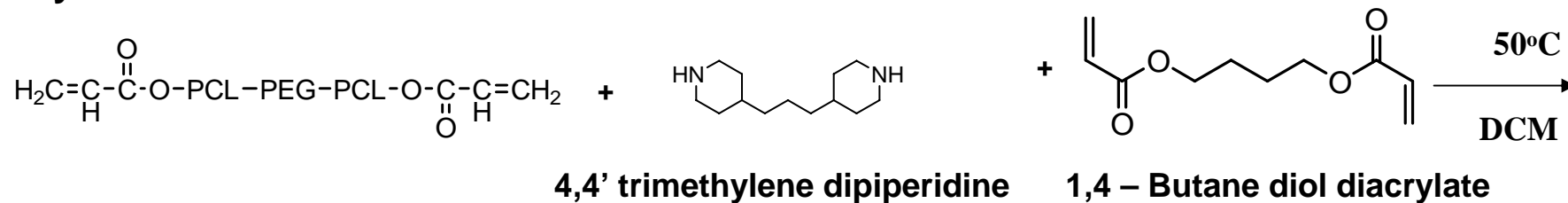
1. Synthesis of PCL-PEG-PCL triblock polymer



2. Synthesis of PCL-PEG-PCL– diacrylate

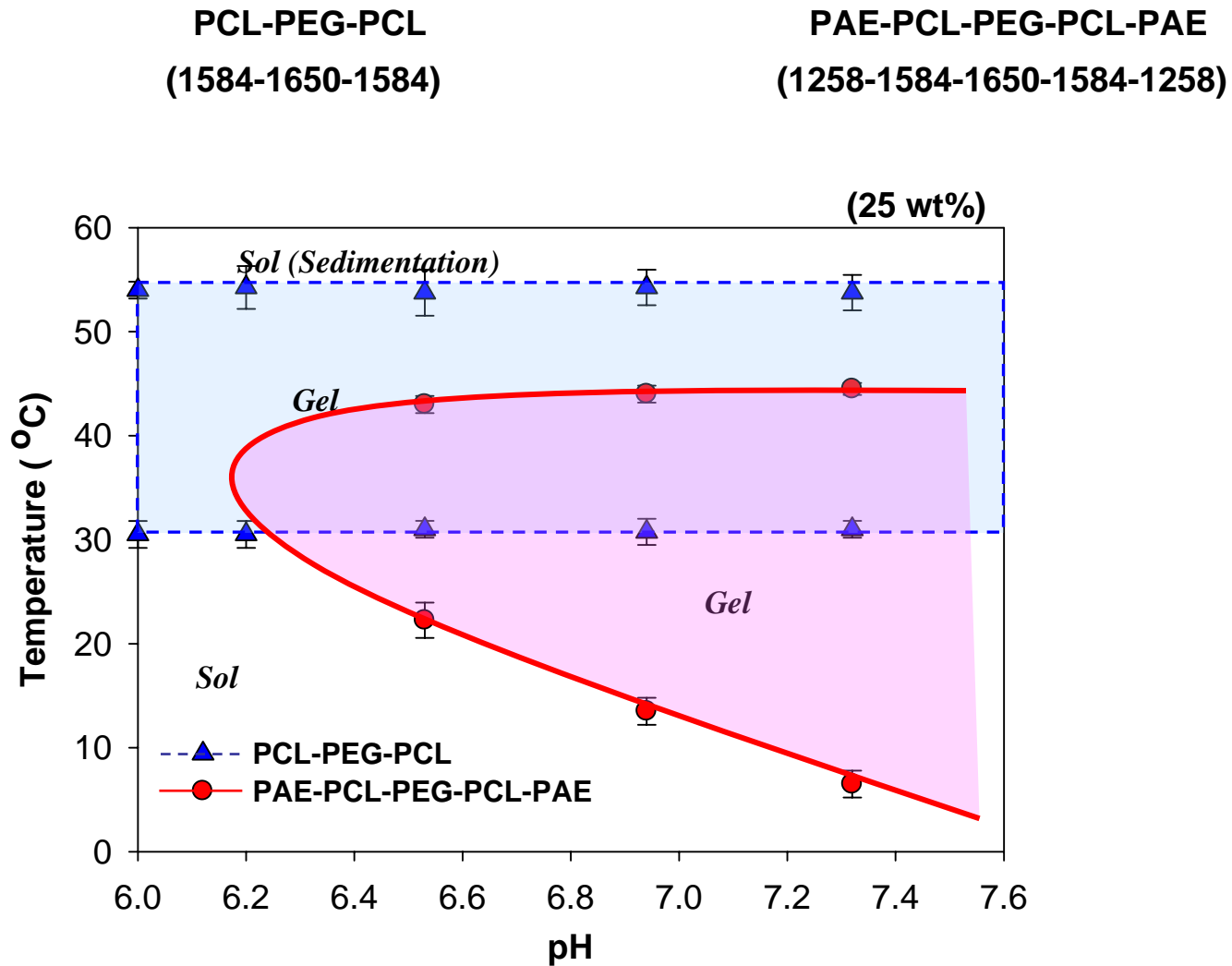


3. Synthesis of PAE-PCL-PEG-PCL-PAE



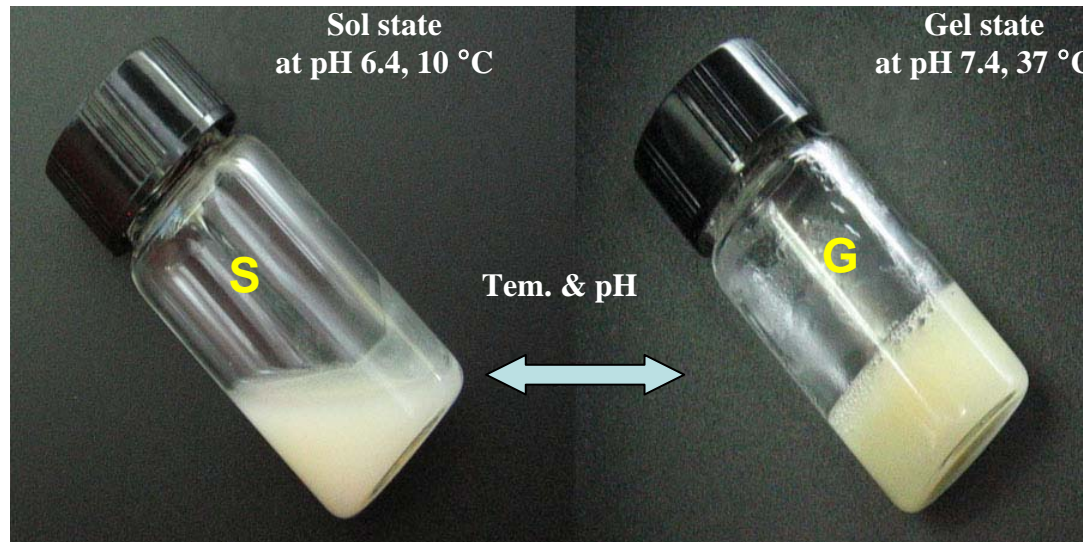
PAE-PCL-PEG-PCL-PAE

• T-Sensitive Gel & pH/T-Sensitive Gel



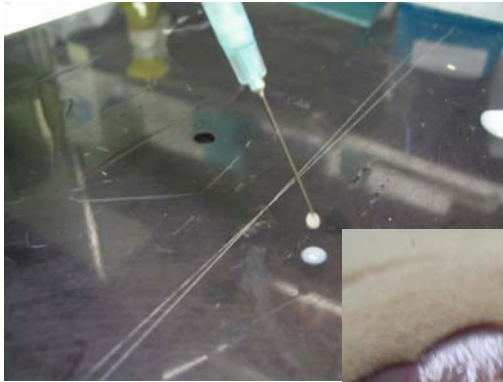
● Sol-gel transition

PAE-PCL-PEG-PCL-PAE (PEG1.65k; PCL/PEG~1.8; PAE~1.25k)



So-Gel phenomena

● Injection to Rat



Sol state
(pH 8.0, Room temp)



Injection to Rat



Gel formation after minutes

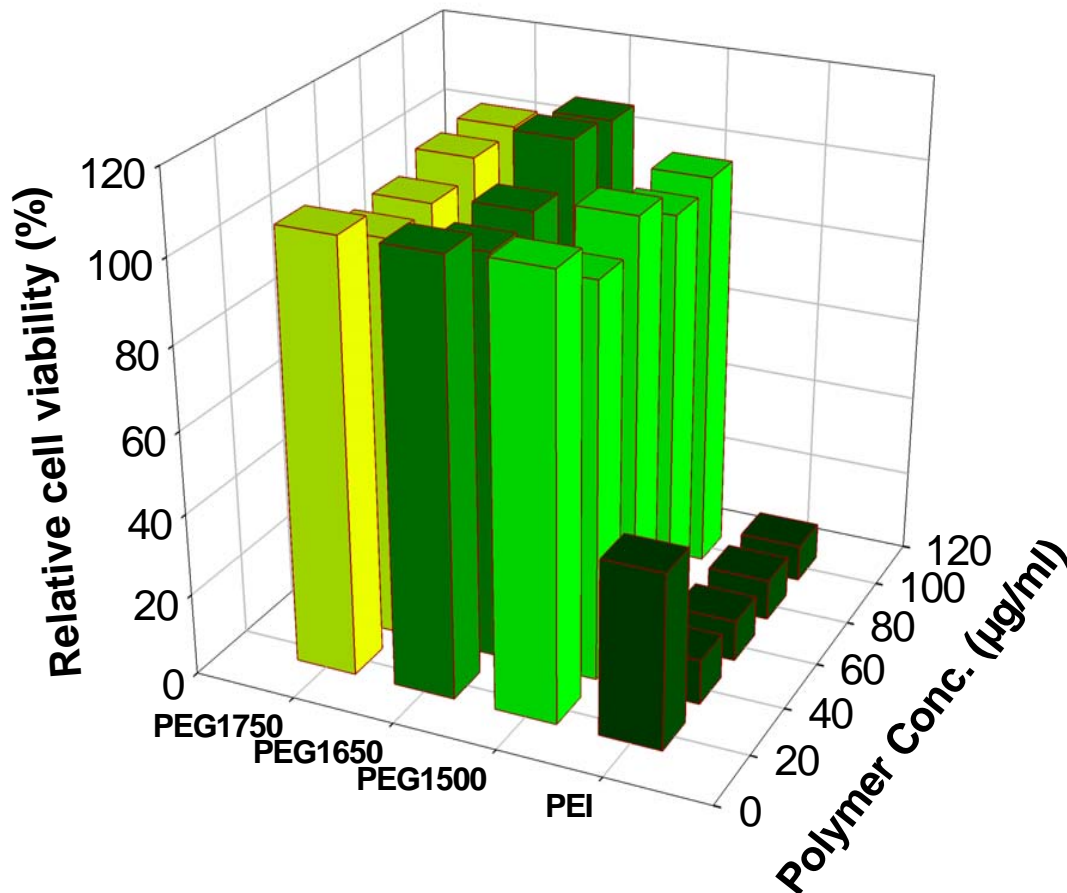


Injected sites

Cytotoxicity of PAE-PCL-PEG-PCL-PAE in vivo test



PAE-PCL-PEG-PCL-PAE (PCL/PEG~1.5; PAE~1.25k)



* **Cell line:** NIH 3T3 (fibroblast).

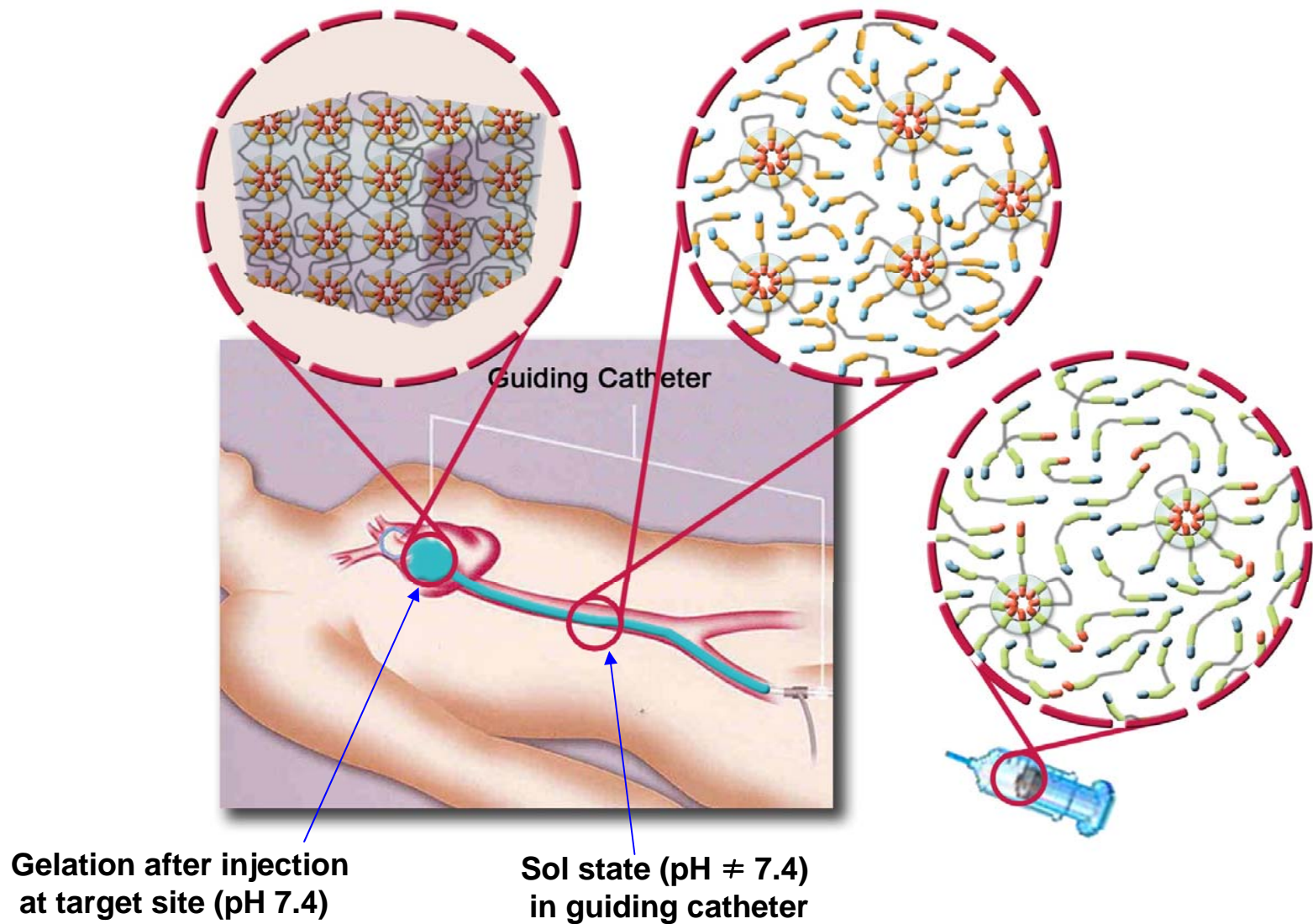
* **Growth medium:** DMEM
(90% Dulbecco's modified Eagle's medium, 10% fetal calf serum, penicillin 100 units/mL, streptomycin 100 µg/mL).

* **XTT assay**

(XTT :2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide)

* **96-well plates, incubator. Microplate reader.**

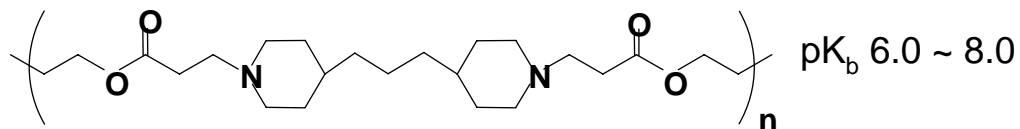
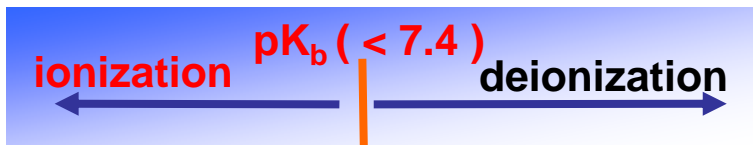
- **Slow Injection Possible to Inside the Body (Cathetering)**



- Cationic Hydrogel as a Depot for Protein Delivery Experiment

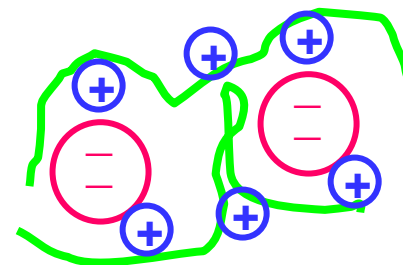
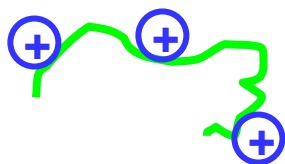
• pH Sensitive Moiety and How to Work

Poly(β -amino esters) - Basic moiety



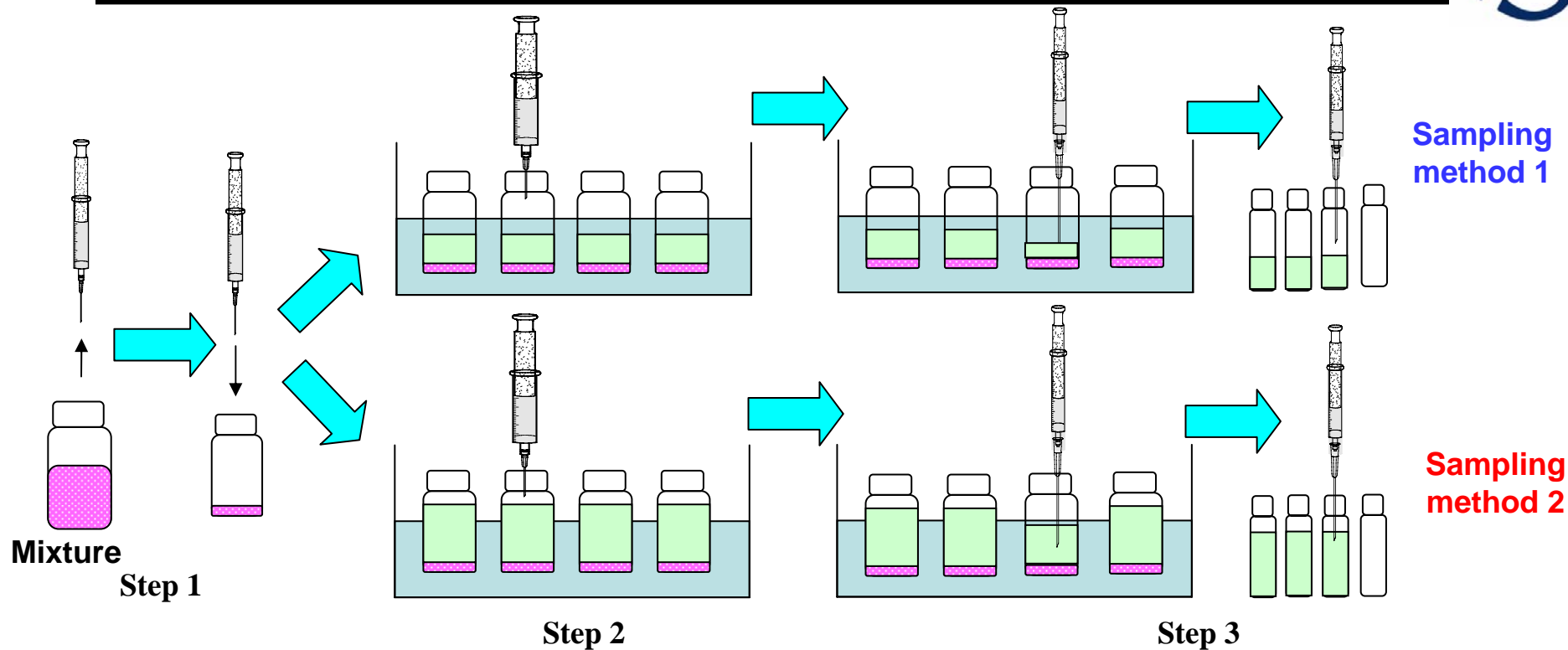
Drug loading by ionic complex

at below pH 7.4



- Insulin as a model anionic protein

● Insulin Loading and Release - *in vitro*

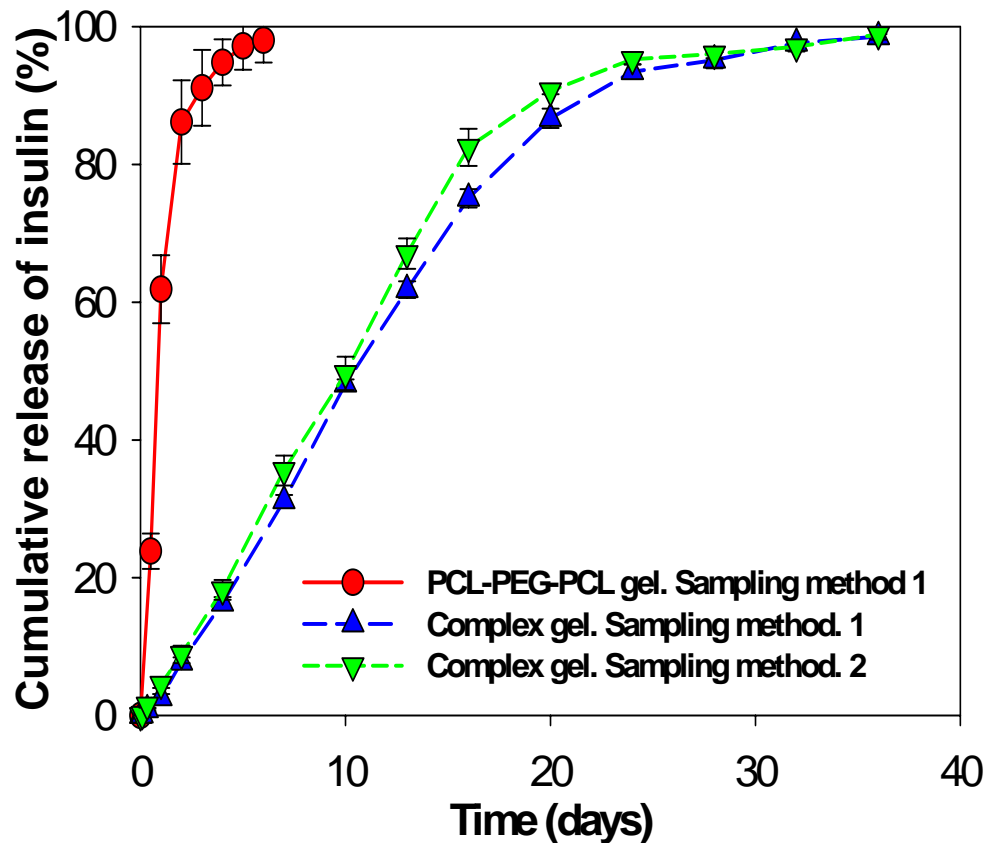


1. 0.5 ml of the complex mixture at pH 7.4 is placed in a 6 ml vial
2. The sample vials were incubate at 37 °C for 30 min, at 37 °C is added to the vial samples.
3. Sampling the insulin release to serum by two methods:
 - **Method 1:** The amount of beginning fresh serum was 3 ml. At a given time, 1.5 ml of the serum in vials (releasing sample) was extracted from the vial samples, and 1.5 ml of fresh serum was supplemented.
 - **Method 2:** The amount of beginning fresh serum was 6 ml. At a given time, the releasing sample was 3 ml, and fresh serum supplemented was 3 ml.

• Effect of Ionic Complex on *in vitro* release

Triblock and pentablock (PEG 1.65k; PCL/PEG~1.8/1; PAE~1.25k)

5 mg/ml insulin in copolymer solution (20%)

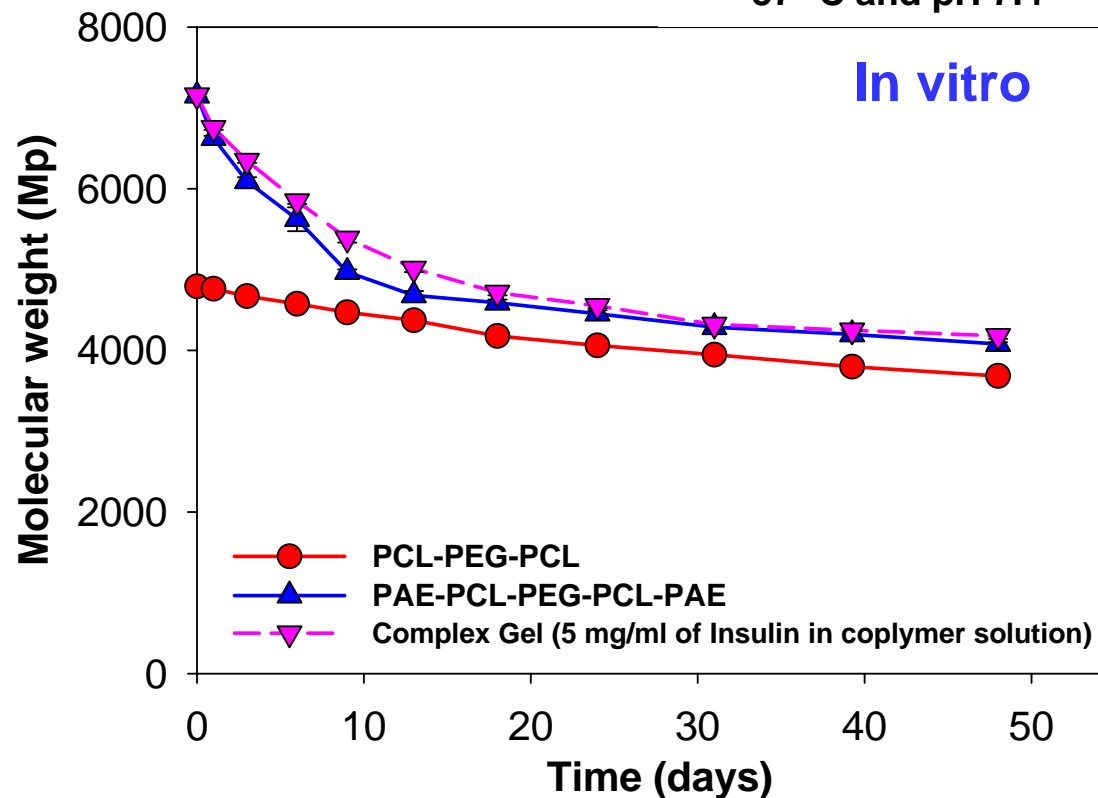


● Effect of Ionic Complex on Degradation

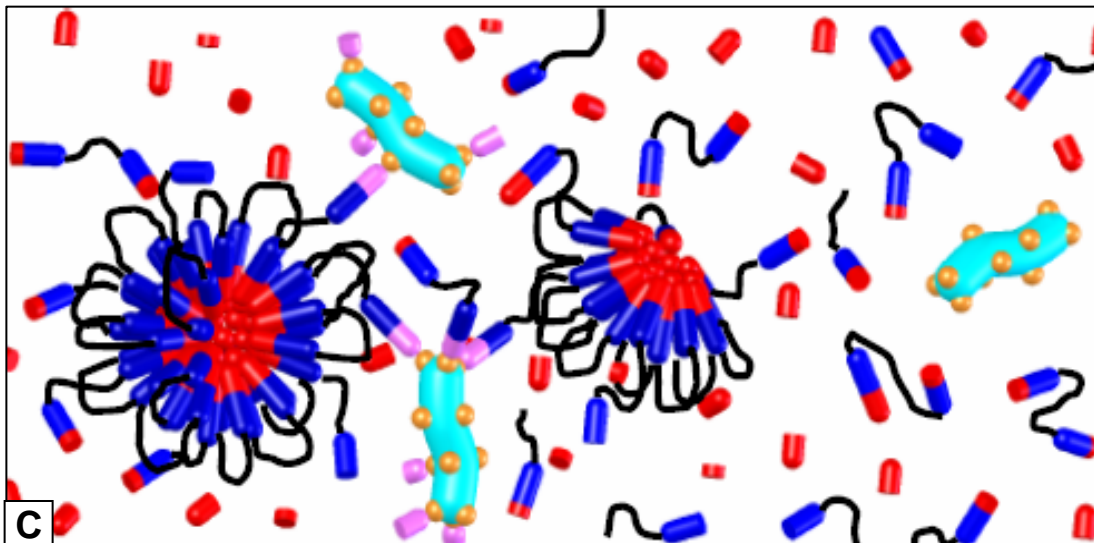
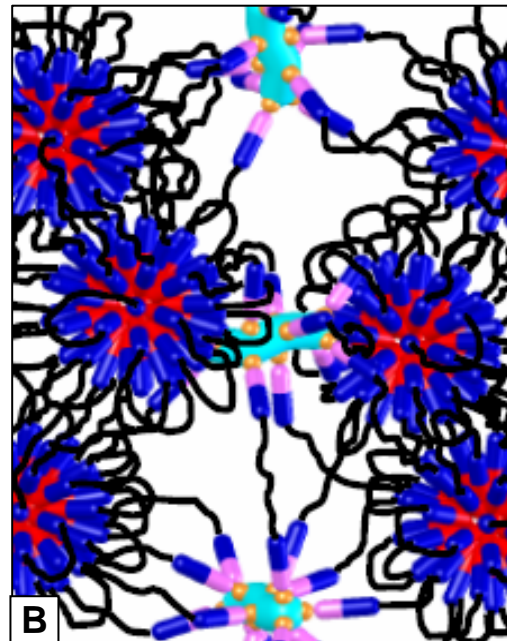
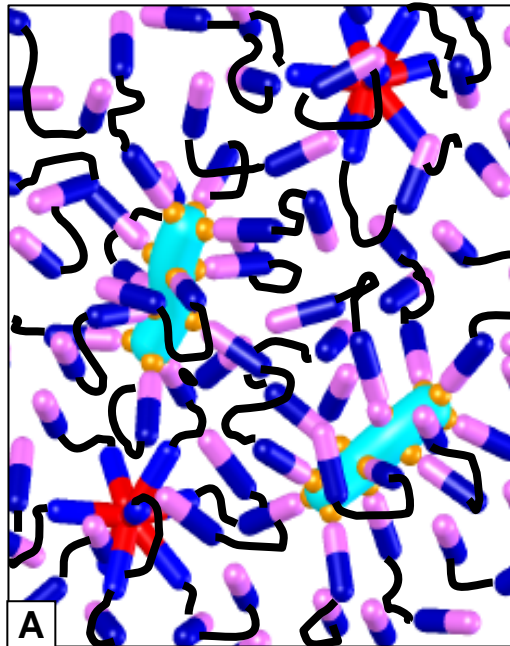
(PEG1.65k; PCL/PEG~1.8/1; PAE~1.25k)
copolymer solution (20%)



37 °C and pH 7.4



Controlled Release Mechanism of pH Sensitive Hydrogel



PAE-PCL-PEG-PCL-PAE at high pH and high temperature

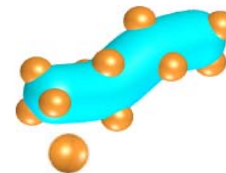
PAE-PCL-PEG-PCL-PAE at low pH and low temperature

PEG hydrophilic

Amino ester ionized

Amino ester de-ionized hydrophobic

PCL hydrophobic



Insulin

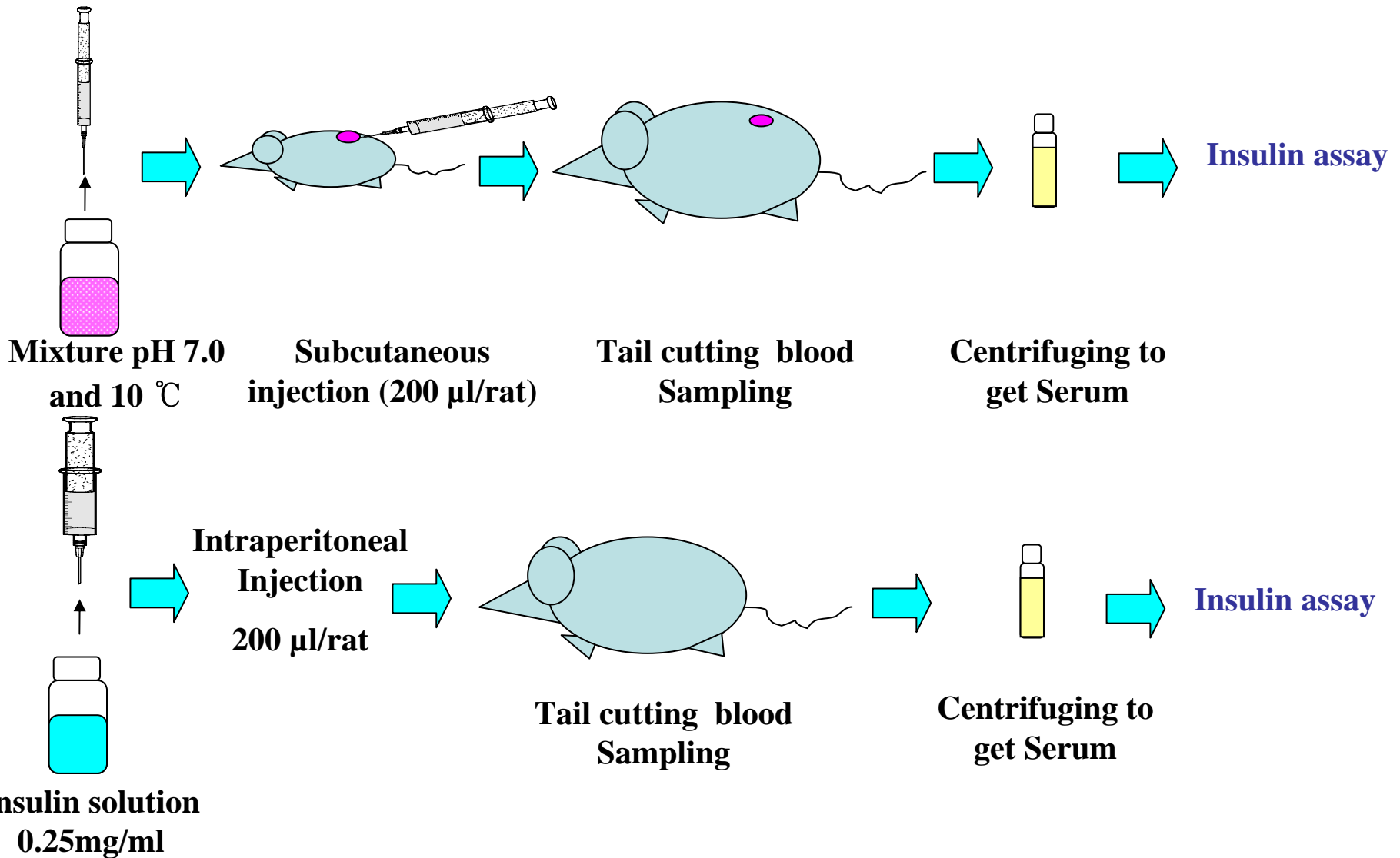
Negative charge on Insulin

A. Copolymer solution with insulin at 10°C and pH 6.0

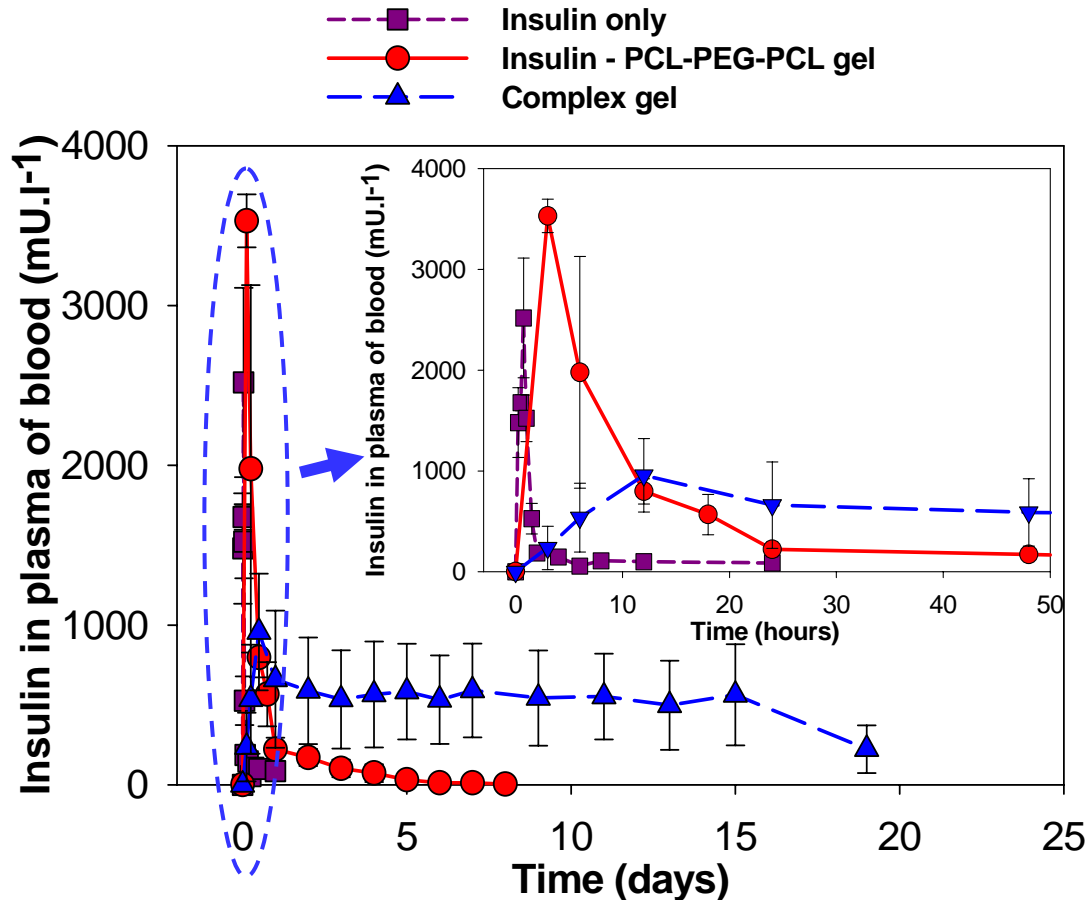
B. Complex gel of copolymer and insulin at 37°C and pH 7.4

C. Insulin releasing depend on the degradation of copolymer at 37°C and pH 7.4

• Insulin Release *in vivo* on SD rats

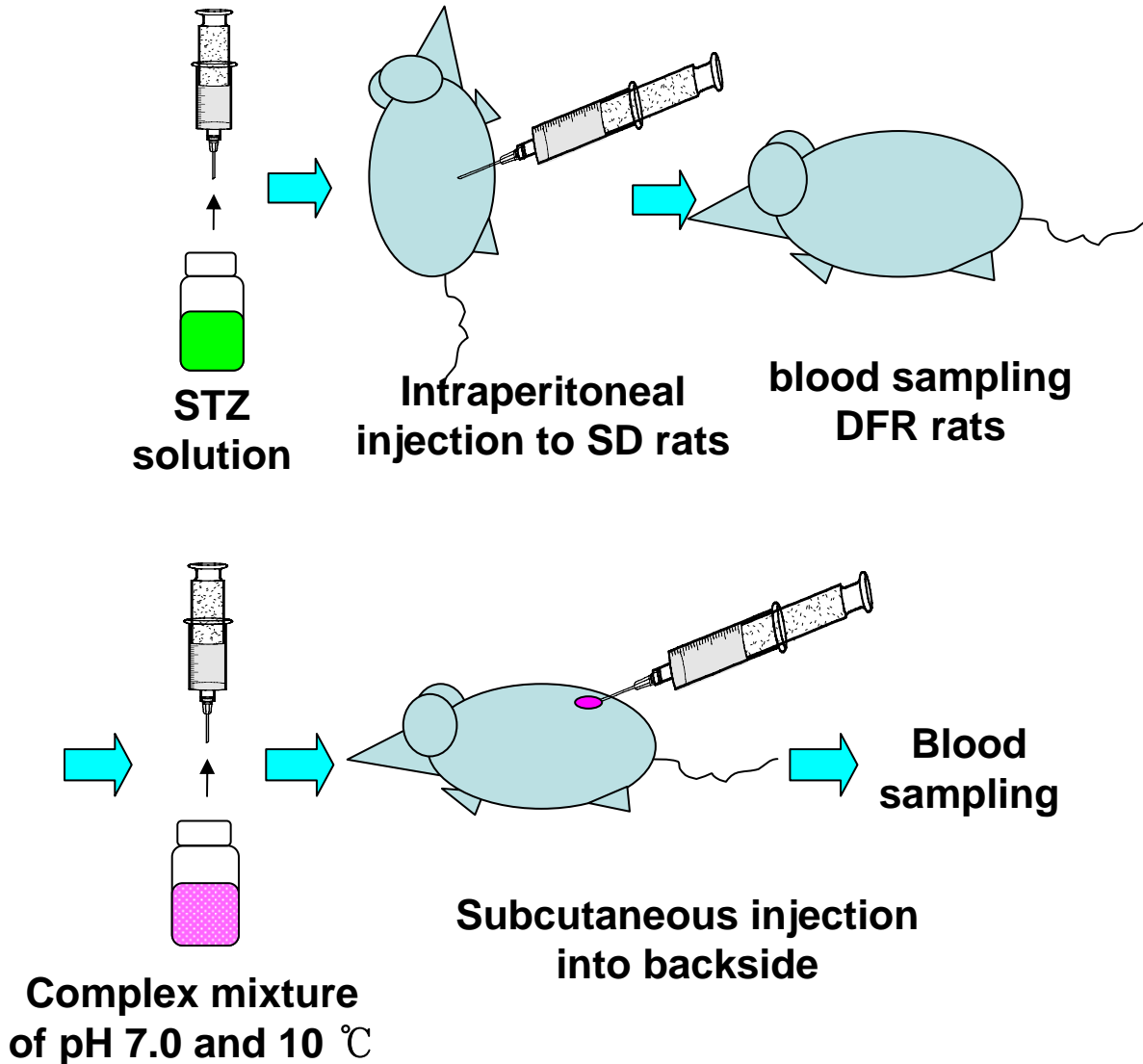


• Insulin Release *in vivo* on SD rats



- **PAE-PCL-PEG-PCL-PAE**
(PCLA/PEG~1.8/1; PEG= 1650)
PAE~ 1250
- **Copolymers conc. : 25wt%.**
- **Insulin formulation 5 mg.ml⁻¹,
200μl/rat.**
- **Insulin conc.: 0.25 mg.ml⁻¹ (in pH
7.4 PBS buffer), 200μl.
(intraperitoneal injection)**
- **Blood sampling from the rat tail
vein.**

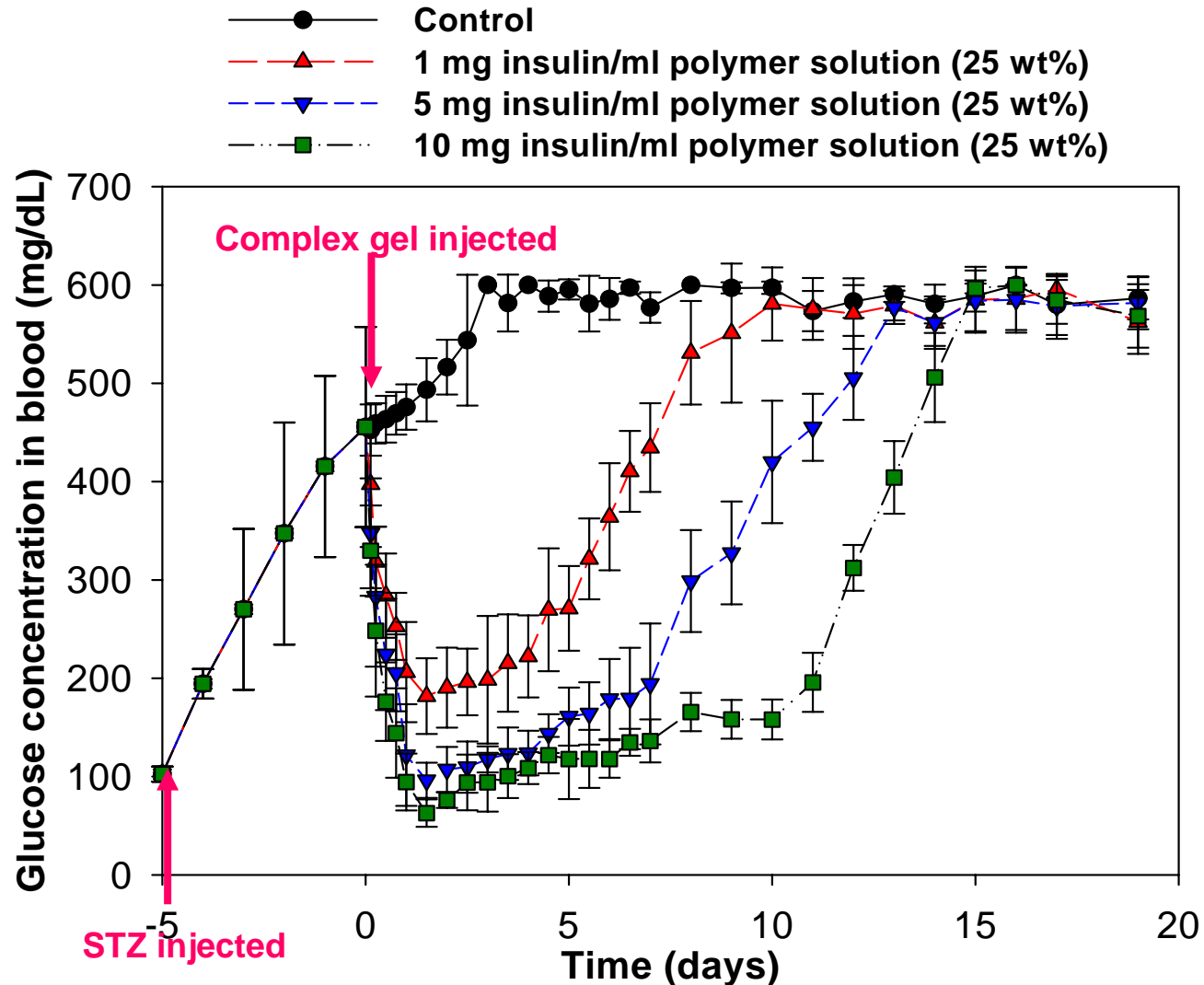
• Insulin Releasing *in vivo* on DFR rats



- **Streptozotocin (STZ) injected: 60 mg.kg⁻¹.**
- **PAE-PCL-PEG-PCL-PAE** (PCLA/PEG~1.8/1; PEG= 1650) PAE~ 1250
- **Copolymers conc. : 25 wt%.**
- **Insulin in the complex mixture 1-10 mg.ml⁻¹**
- **n = 5**
- **Complex mixture injected: 200µl.**
- **Blood sampling from the rat tail vein.**

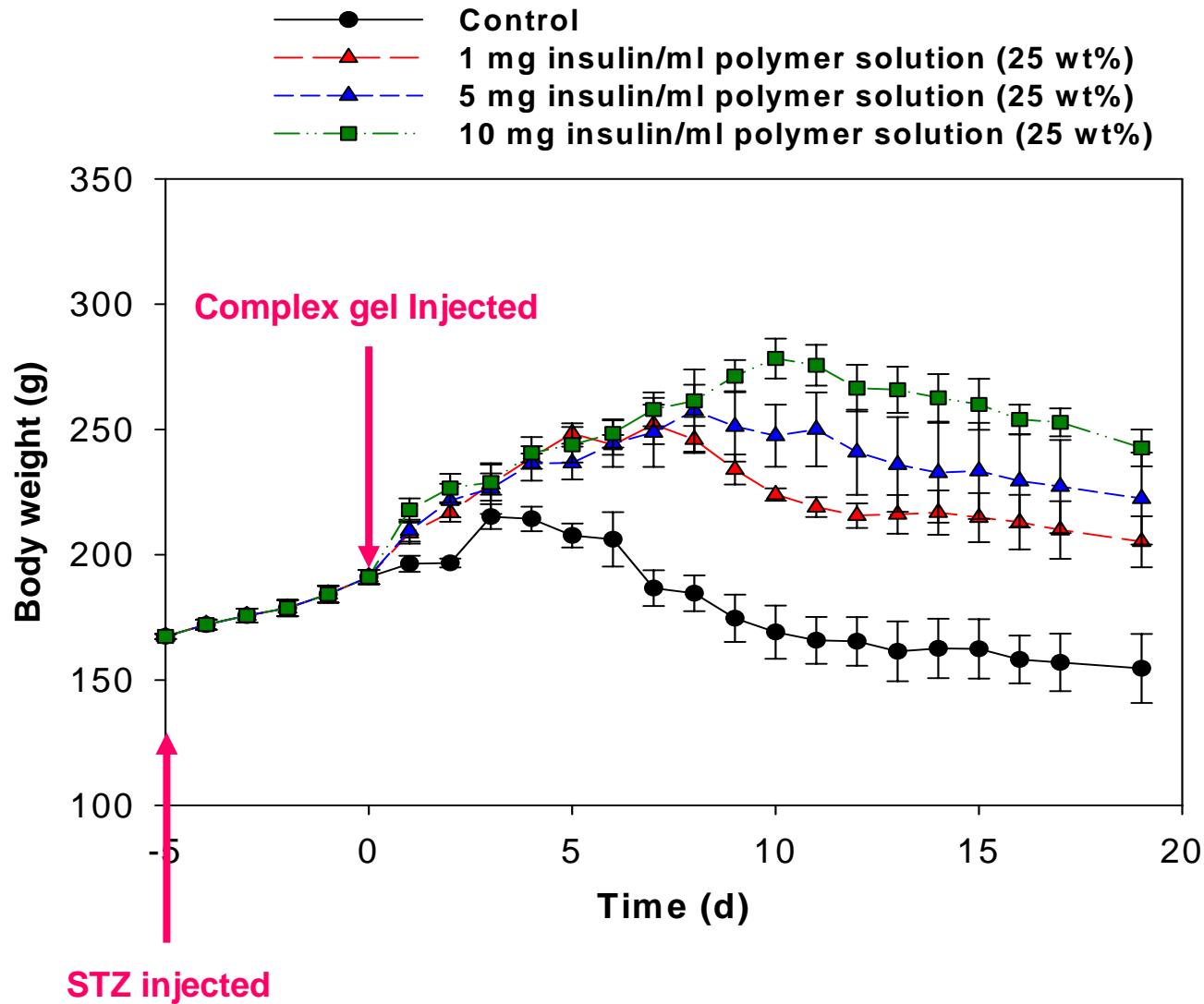
● Glucose Concentration in Blood of DFR Rats

Glucose concentration in blood of diabetic rat with insulin-hydrogel complex



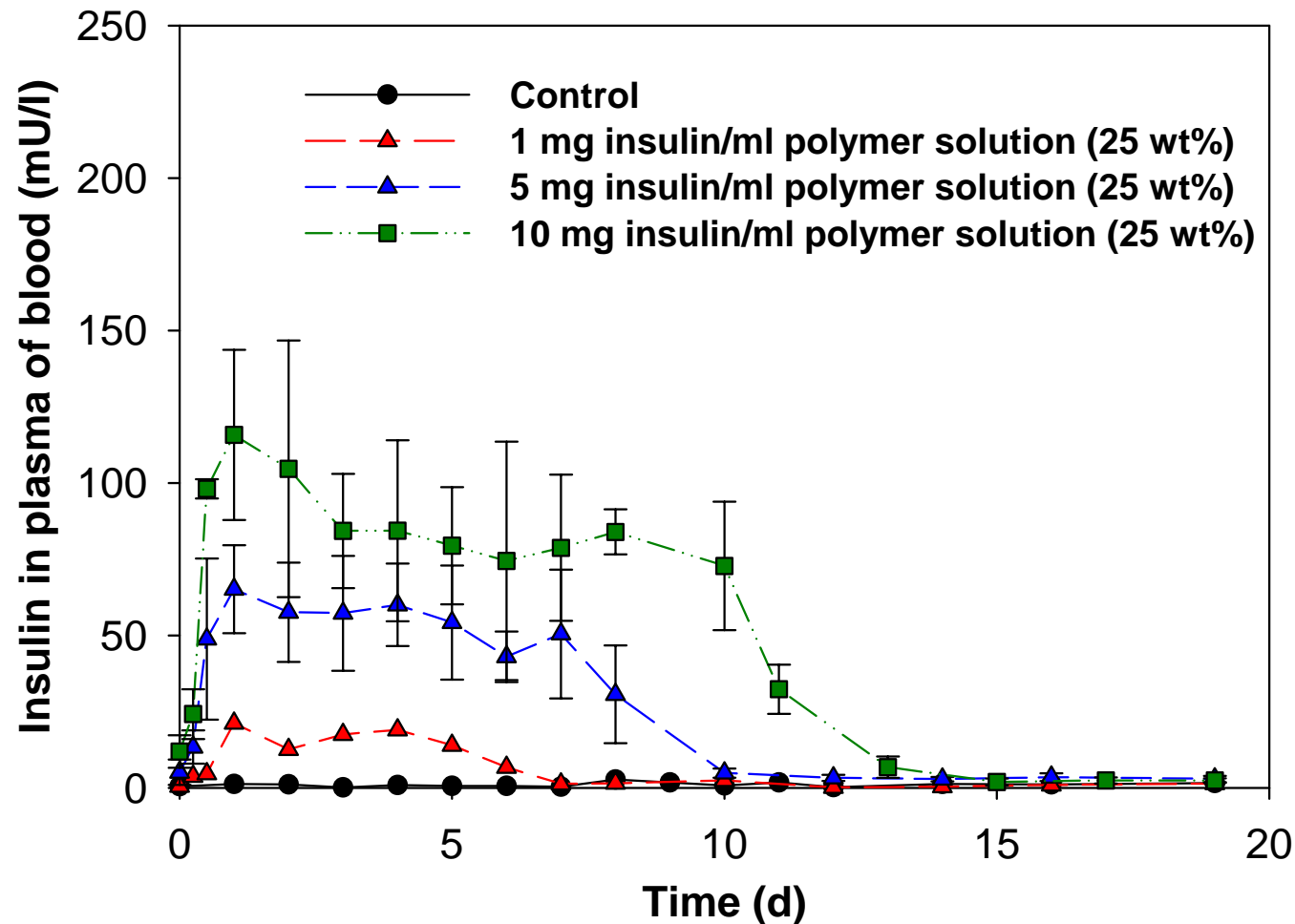
• Body Weight Change of DFR Rats

Body weight of diabetic rat with insulin-hydrogel complex



● Insulin in Plasma of Blood of DFR Rats

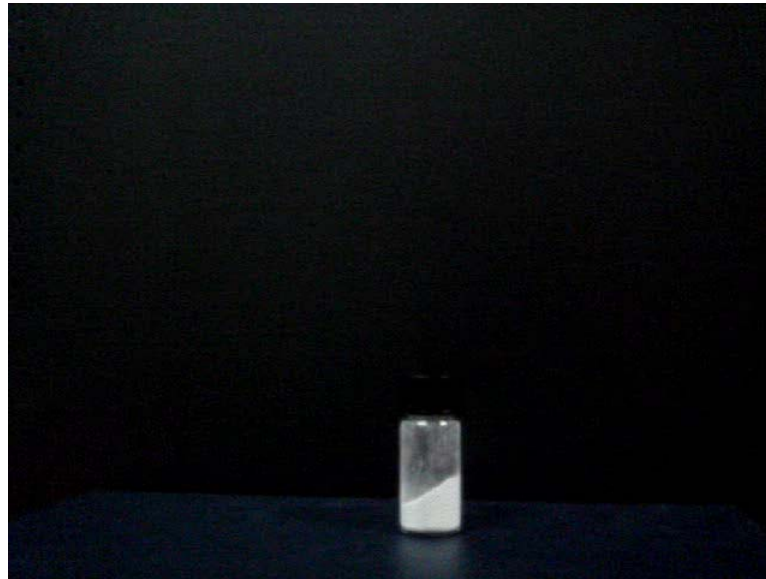
Insulin concentration in blood of Diabetic rat



● Possible Drug Candidates

- **Protein/peptide drugs are increasingly important**
 - **Diabetes** (Insulin, Symmlin, Exendin, Somatokine)
 - **Cancer** (Interferon, Monoclonal Antibodies, Vaccines)
 - **Cardiovascular Drugs** (Natreacor, GPIIB receptor, Protein G receptor)
 - **Inflammation** (TNF-a, IL1-RA)
 - **HIV/AIDS** (Somatostatin, T20, T1249, IL-2, Interferon)
 - **Human Growth Hormone**
 - **Gene Delivery**

• Insulin Complex Powder

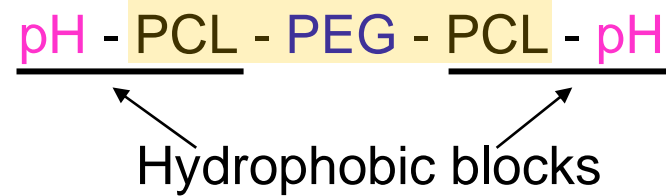


The complex powder could be dissolved within 5 minutes

• Two Types of pH Sensitive Moiety

- pH sensitive moiety
needs high sensitivity

Temperature sensitive blocks



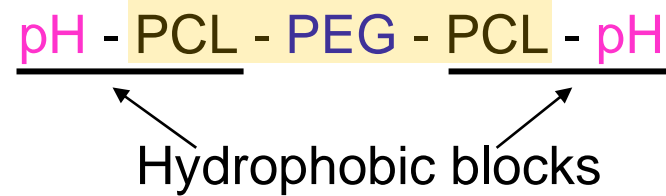
Basic moiety : pK_b (6.2 - 7.5)

- **Backbone** amine group
- **Pendent** amine group
(Tertiary & secondary amine)
- **Cationic charge**
- **Complex with anionic drug**
- **Screening work**
→ **Poly(amino acid) derivatives**
Poly(amido amine) derivatives

• Two Types of pH Sensitive Moiety

- pH sensitive moiety
needs high sensitivity

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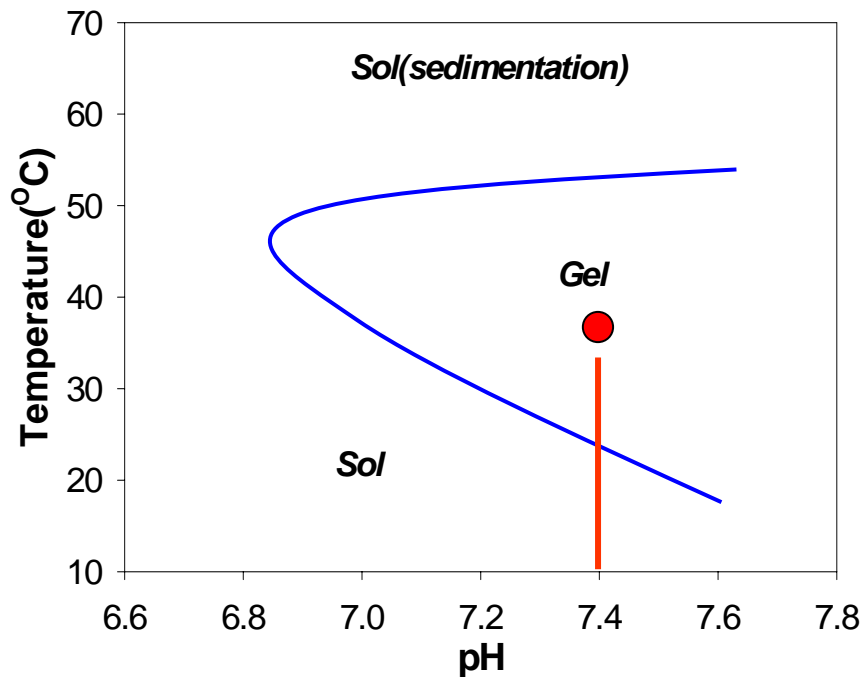
Acidic moiety : pK_a (7.4 - 7.8)

- **Acid group**
(-COOH, -SH, -SO₂NH-)
- **Anionic charge**
- **Complex with cationic drug**
- **Screening work**
→ Sulfonamide derivatives

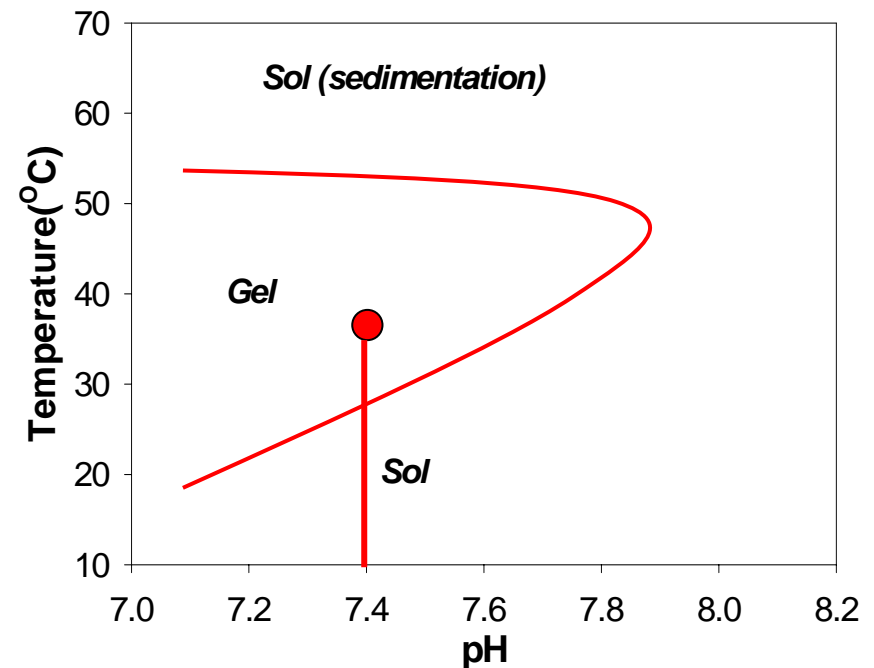
• Two Types of pH & Temperature Sensitive Hydrogel



Cationic hydrogel with basic moiety



Anionic hydrogel with acidic moiety



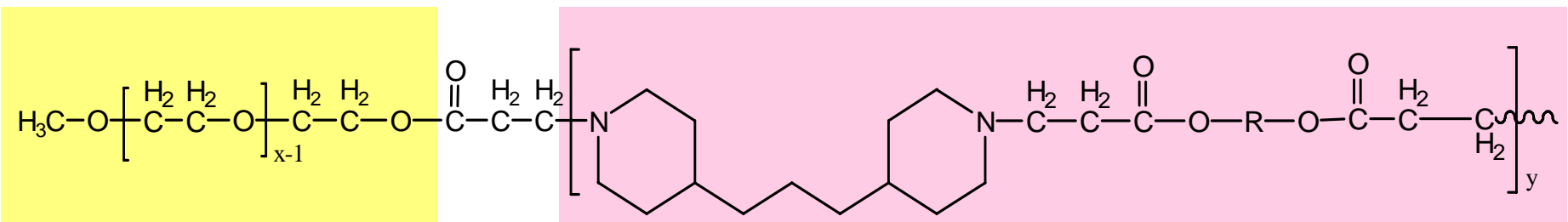
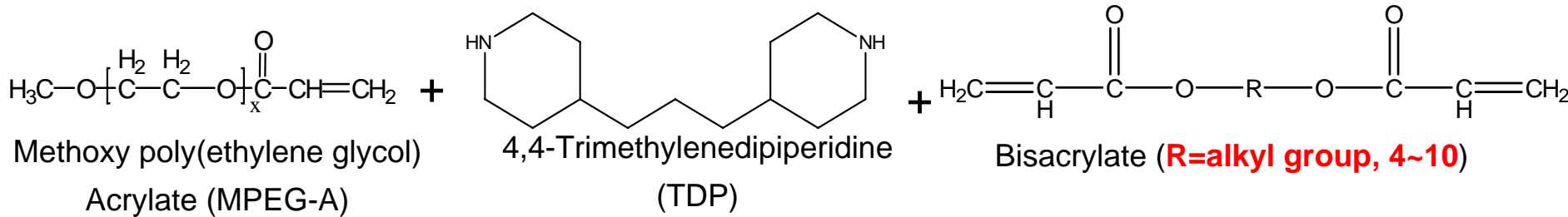
Conclusions

Main characteristic of this hydrogel

- 1) **Injectable biodegradable pH- and temperature-sensitive hydrogels**
- 2) **No clogging during injection (cathetering is possible)**
- 3) **No burst release of ionic proteins and drugs**
- 4) **Powder form (Type II)**
- 5) **Easy to dissolve**
- 6) **No cytotoxicity**
- 7) **A little pH change during degradation**

pH Sensitive Micelles

Synthesis:



MPEG-Poly(β -amino ester) block copolymer

MPEG-PAE

Hydrophilic block

pH-sensitive block

Polymer prepared

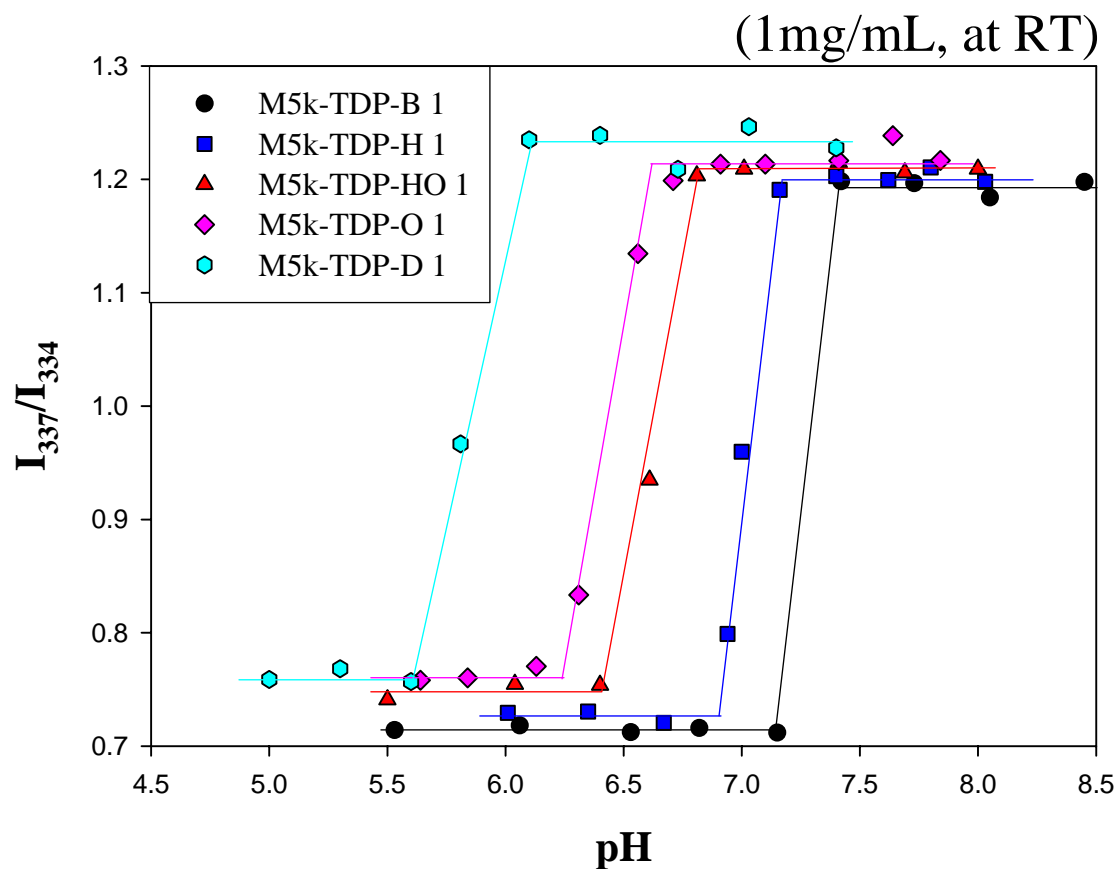
Sample name	Diamine	Bisacrylate	M _n of PEG	M _n of PAE
PEG5k-P-H6.2K	P ¹⁾	HDA ³⁾	5,000	6,214
PEG2k-TDP-H5.8K	TDP ²⁾	HAD	2,000	5,776
PEG2k-TDP-H2.8K	TDP	HAD	2,000	2,826
PEG5k-TDP-B12K	TDP	BAD ⁴⁾	5,000	12,035
PEG5k-TDP-H1.4K	TDP	HAD	5,000	1,442
PEG5k-TDP-H6.2K	TDP	HAD	5,000	6,153
PEG5k-TDP-O10.2K	TDP	ODA ⁵⁾	5,000	10,182
PEG5k-TDP-D9.8K	TDP	DDA ⁶⁾	5,000	9,837
PEG5k-TDP-HO	TDP	HAD+ODA	5,000	-

M_n : Calculated by GPC

1) Piperazine 2) 4,4'-trimethylene dipiperidine 3) 1,6-hexanediol diacrylate 4) 1,4-butanediol diacrylate

5) 1,8-octanediol diacrylate 6) 1,10-decanediol diacrylate

pH Sensitivity of Block Copolymers

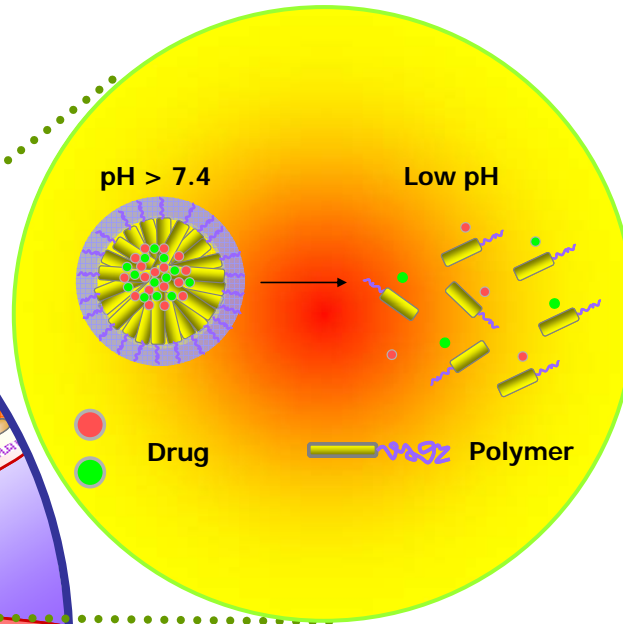
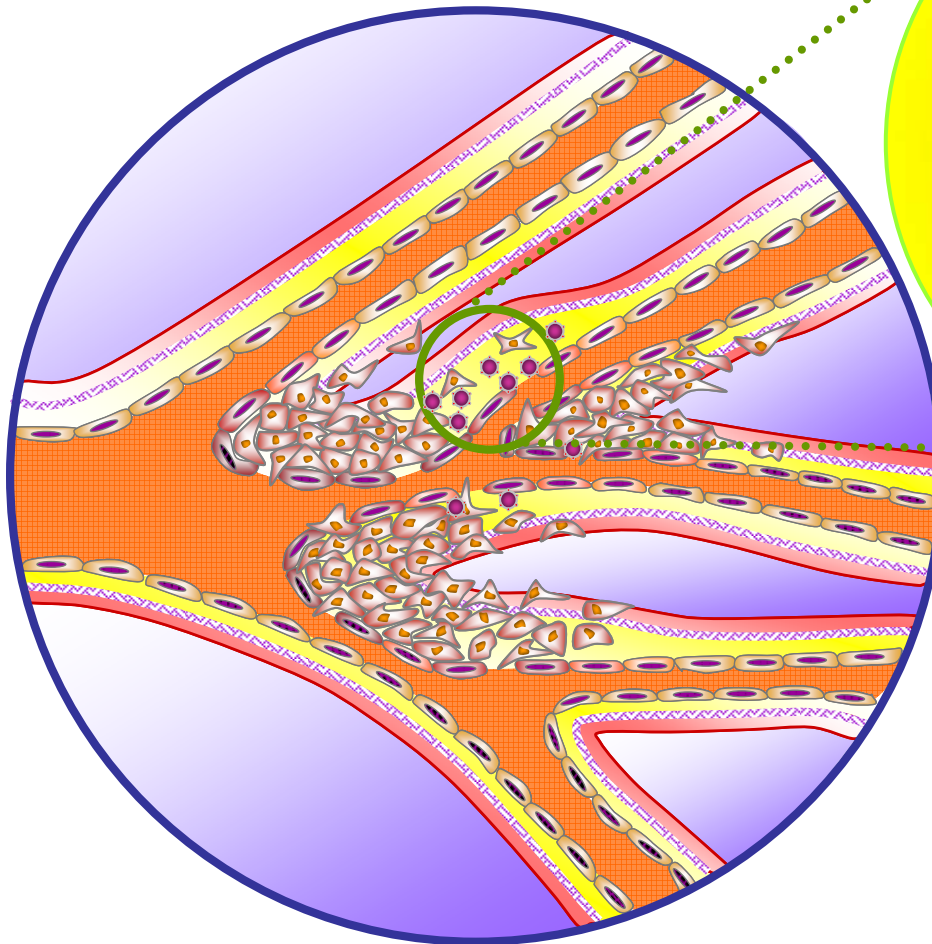


	pH change region
M5k-TDP-B 1	7.15~7.42
M5k-TDP-H 1	6.90~7.15
M5k-TDP-HO 1	6.40~6.81
M5k-TDP-O 1	6.25~6.65
M5k-TDP-D 1	5.60~6.10

Excitation spectra

337/334 band intensity ratio of pyrene

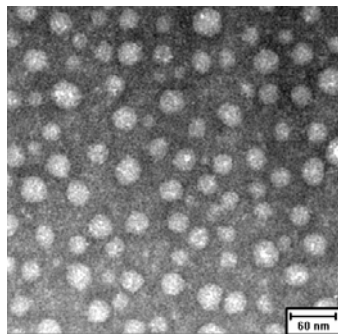
Tumor



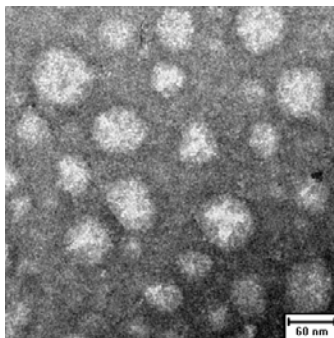
- **Accumulation of Micelles at tumor by EPR effects**
- **Due to low extracellular pH, micelles can be disrupted, and the drug released at tumor site**

Size distribution

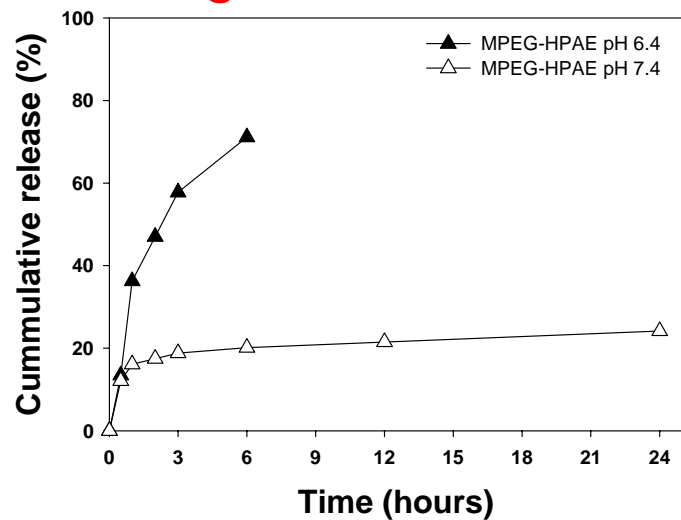
MPEG-HPAE



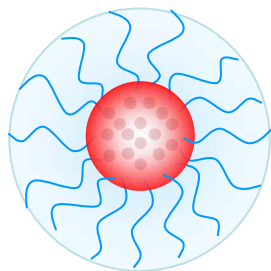
DOX-MPEG-HPAE



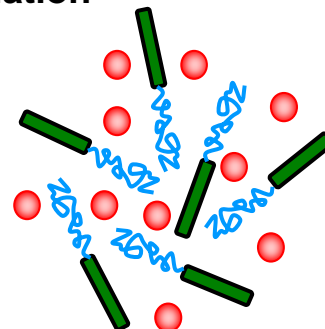
Drug release



Micelle structure



Dissociation



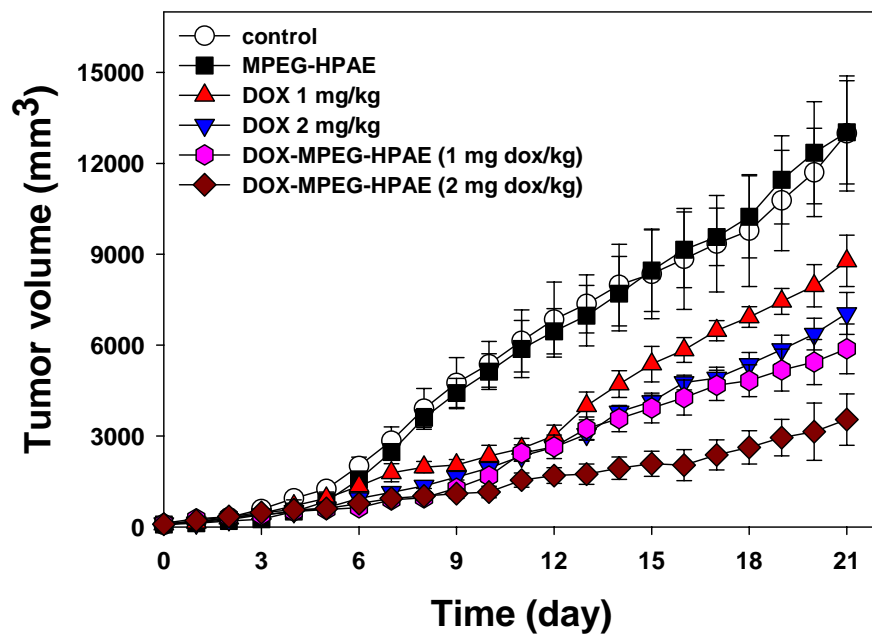
Physiological pH (pH 7.4)

Blood vessel

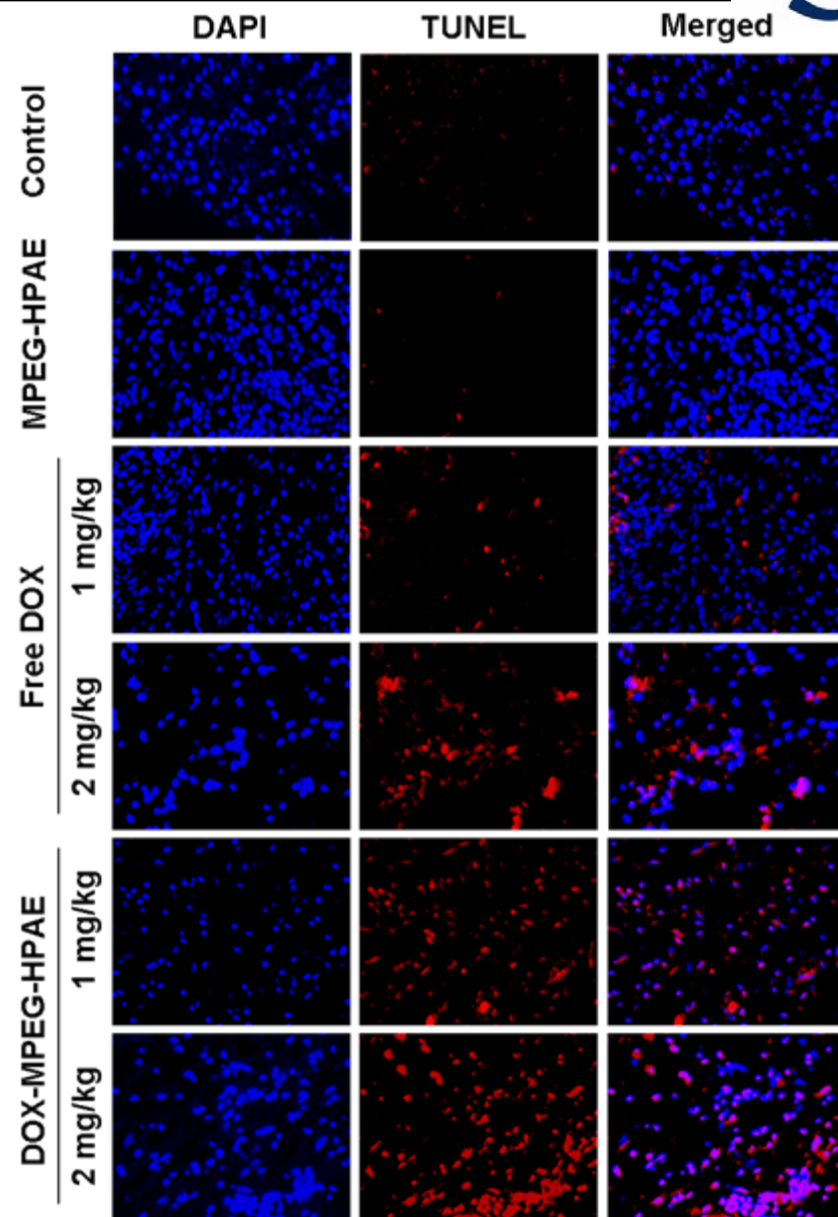
Weakly acidic extracellular pH

Tumor site

In vivo Anti-tumor Effect



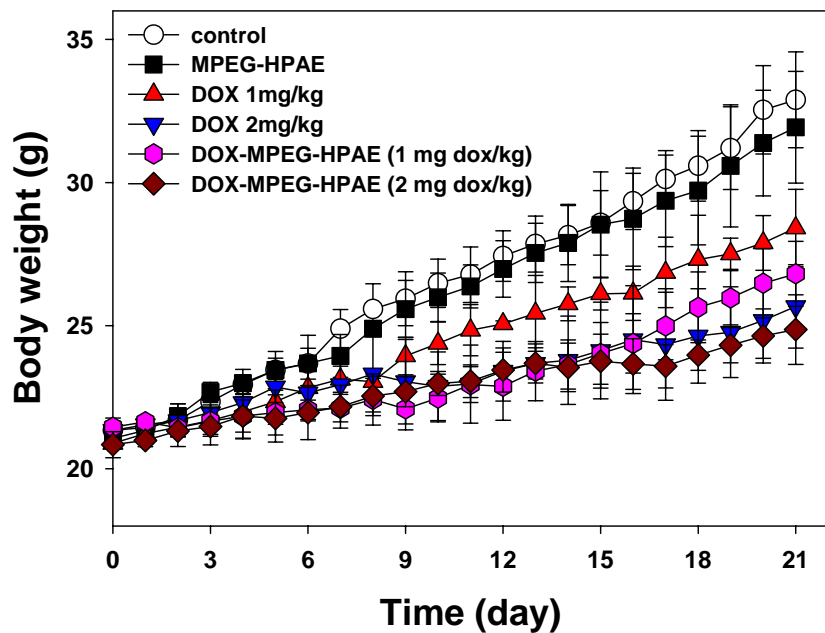
- Male C57BL/6 mice
- Subcutaneous tumors of 1.0×10^6 B16F10 cells
- Drug were injected once every 3day for 21days via a tail vein



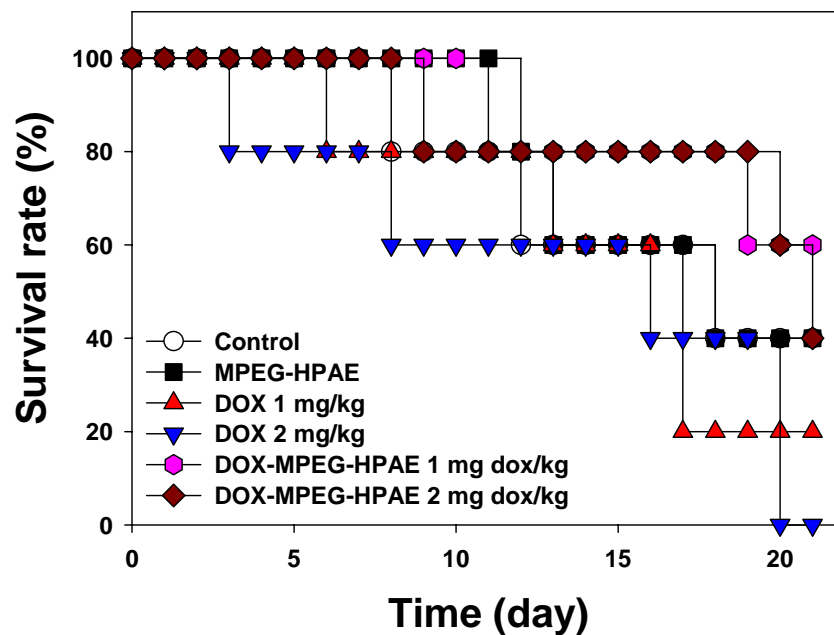
Cellular In vivo anti-tumor effect



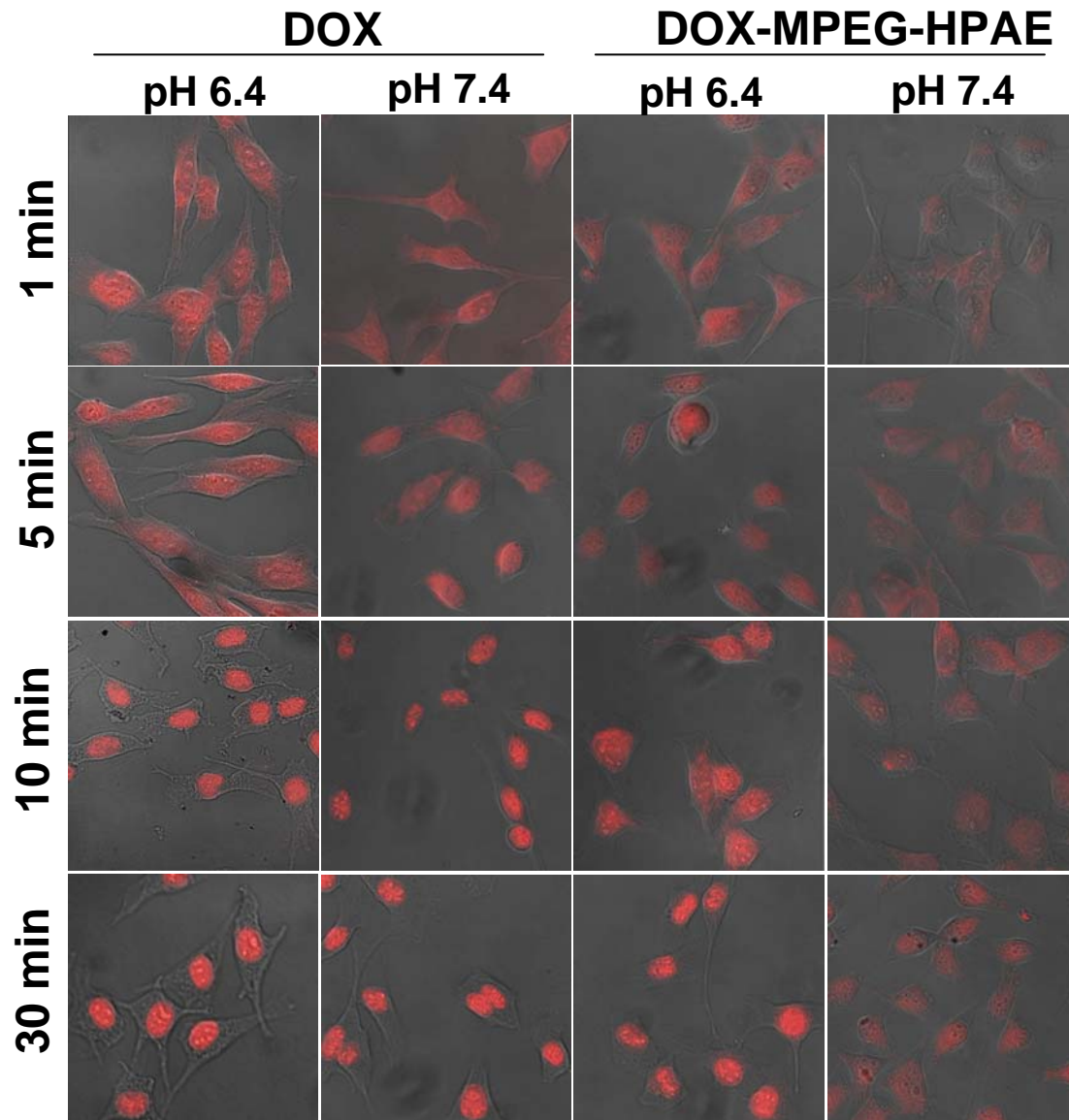
Body weight



Survival rate

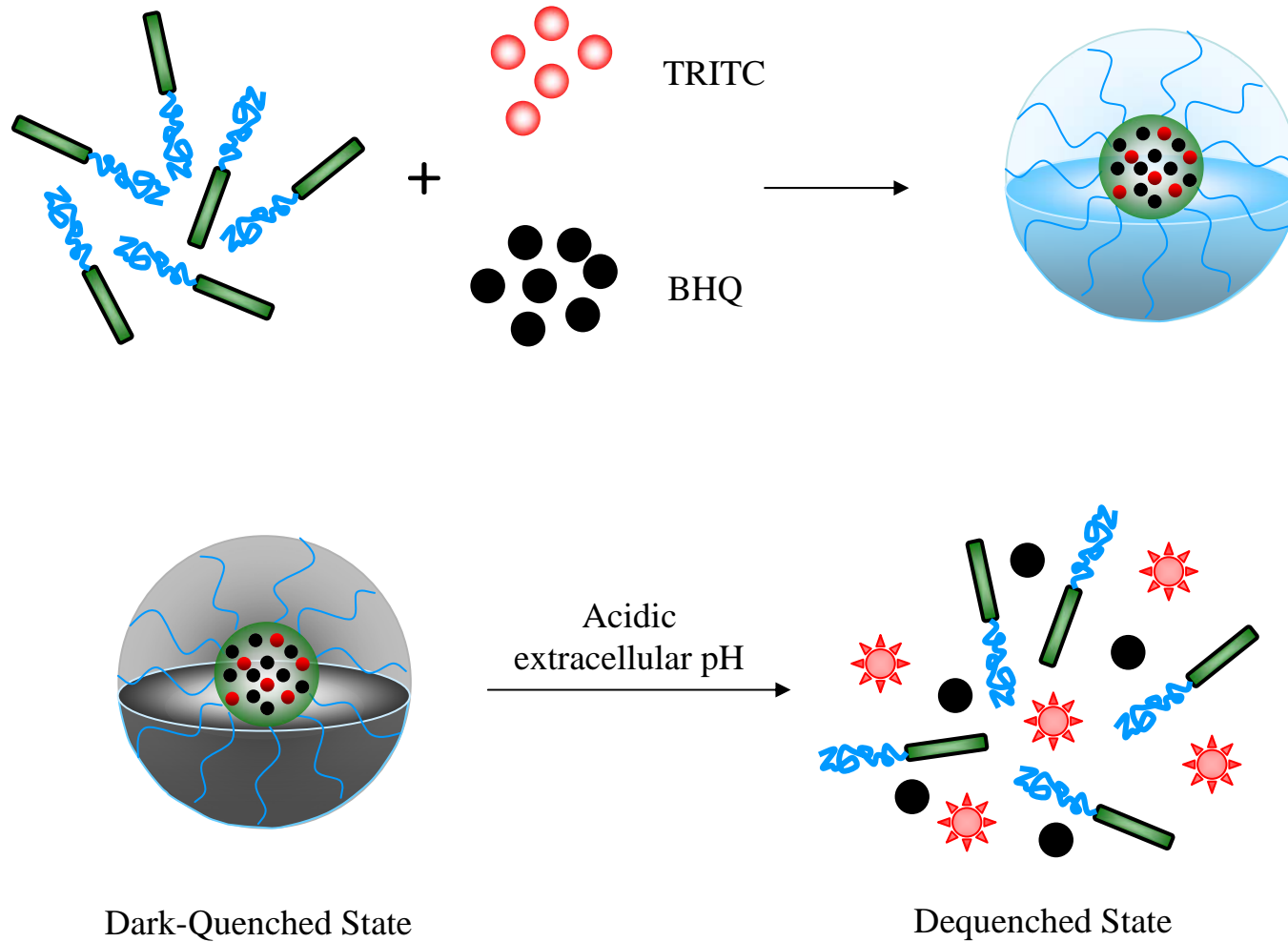


Cellular uptake of DOX

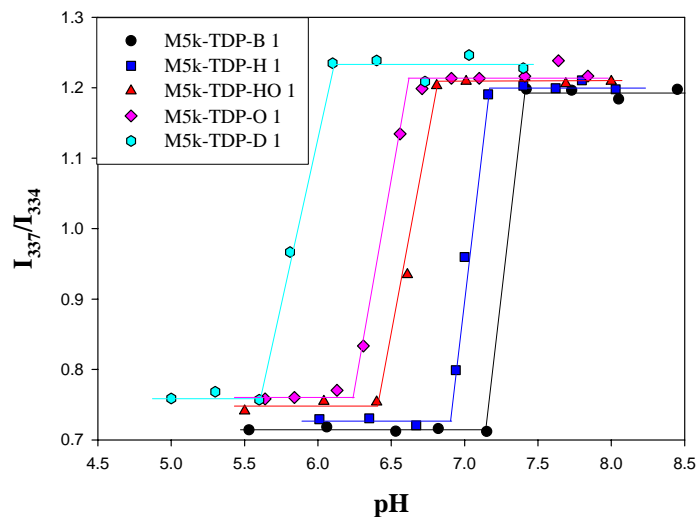


B16F10 cells

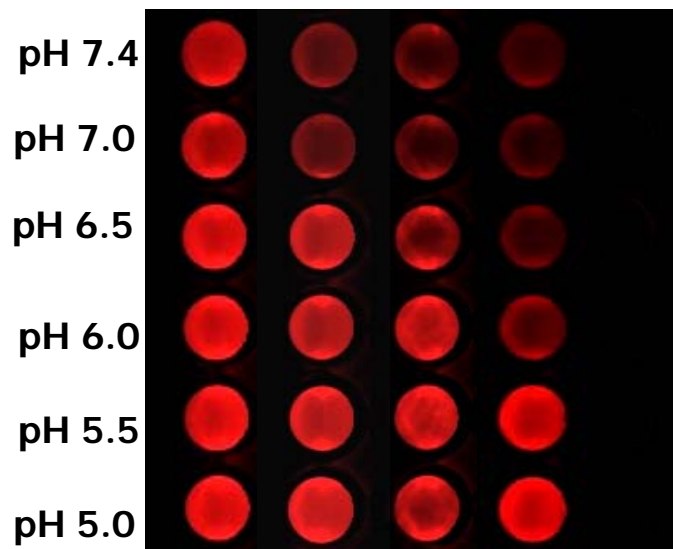
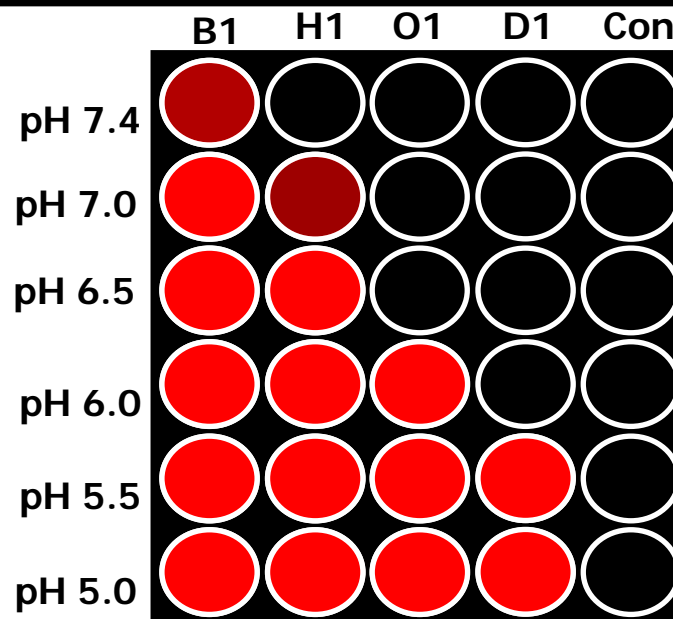
Dark-quenched pH Sensitive Probe



Fluorescent Intensity



	pH change region
M5k-TDP-B 1	7.15~7.42
M5k-TDP-H 1	6.90~7.15
M5k-TDP-HO 1	6.40~6.81
M5k-TDP-O 1	6.25~6.65
M5k-TDP-D 1	5.60~6.10



● Acknowledgements



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