

Smart nanoparticles for drug, gene and nitric oxide delivery



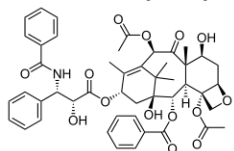
Won Jong Kim, Ph.D

Department of Chemistry
POSTECH

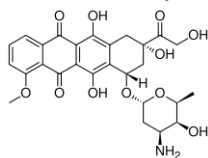
Drug Delivery System

Limitations of small molecular drugs

Paclitaxel (PTX)



Doxorubicin (DOX)



Low efficiency

- Poor solubility and stability
- Renal/hepatic clearance

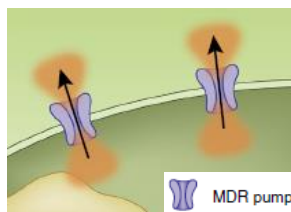
Unfavorable side effects

- Nonspecific distribution
- Hair loss and immunodeficiency

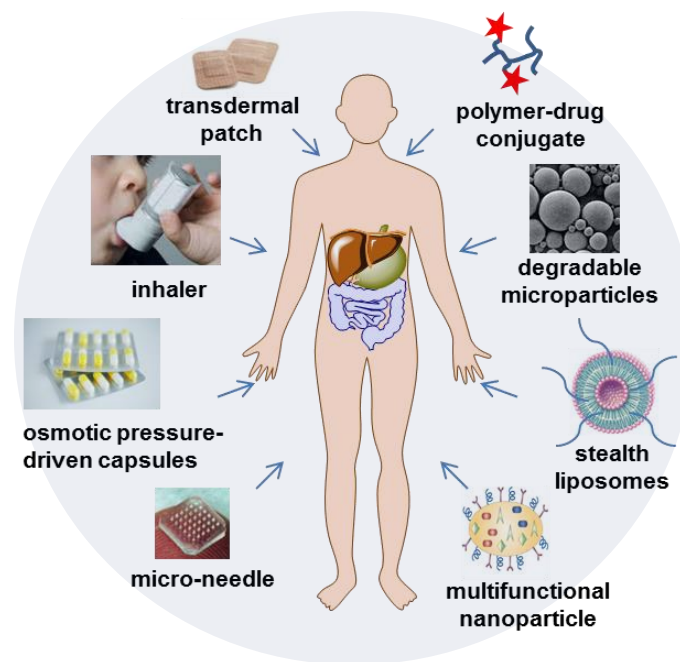


Multidrug resistance

- Active efflux of drugs



Various Drug Delivery Systems



Nanoparticle-based Delivery System

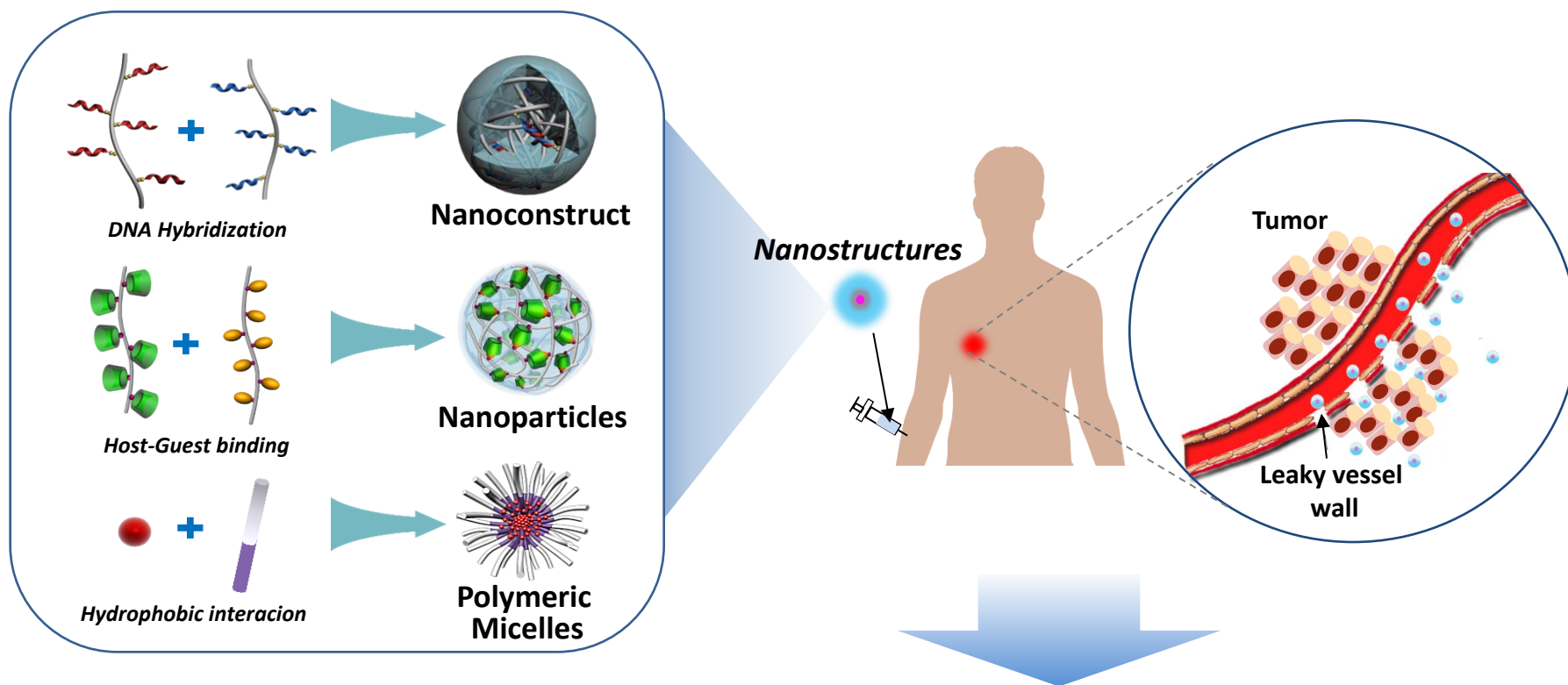


Nanomedicine (Targeted, Programmed)

Ref: *Nat. Biotech.* **2015**, 33, 941-951.

Self-assembled Nanostructures

Self-assembly is a simple and useful mechanism for preparing nanostructure in a complex biological system.

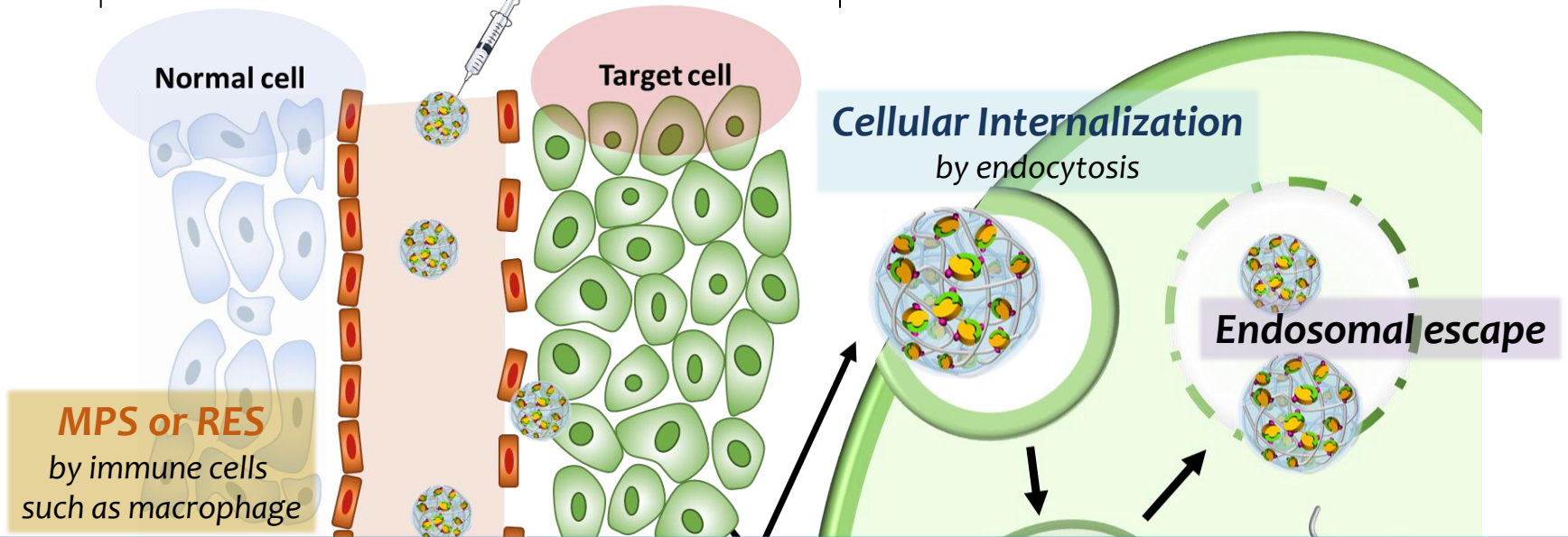


Chemotherapeutic drug delivery system to target site

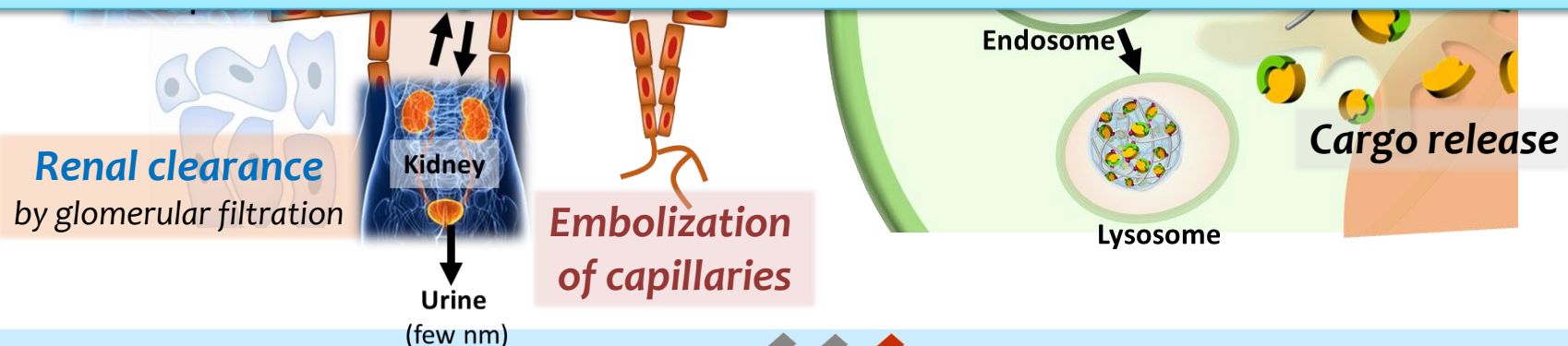
Biological Barriers of Self-assembled Nanomedicine

Extracellular Barriers

Intracellular Barriers

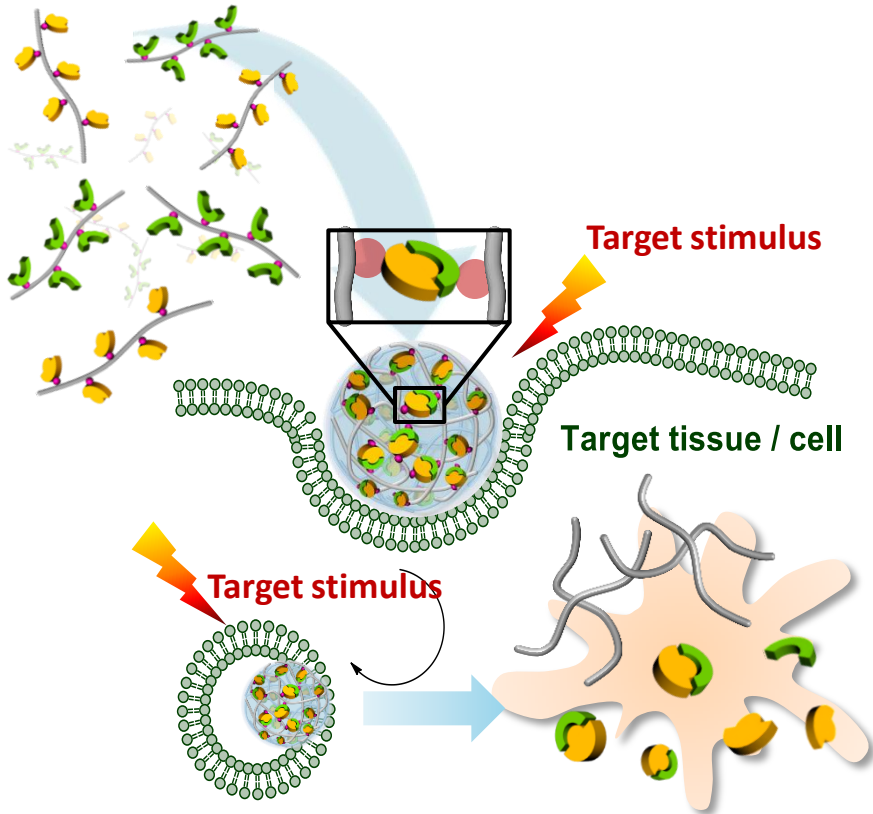


To overcome various biological barriers, **stimuli-regulated intelligent delivery system** is required



Intelligent Drug Carrier: Stimuli-responsive

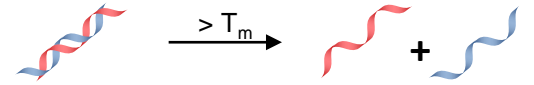
Stimuli-responsive intelligent system



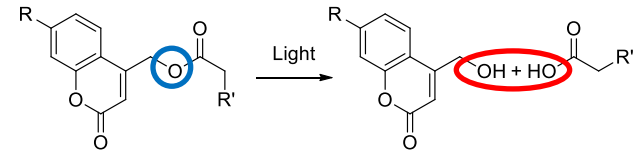
- High accumulation on target site
- Site-specific release for low side-effect and high efficacy

Exogenous stimuli

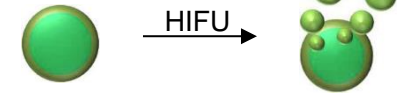
Temperature



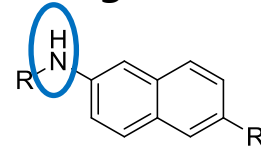
Light



Ultrasound



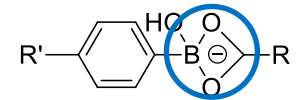
Endogenous specific stimuli



pH



Redox potential



Small molecule

1. Drug delivery by active and passive tumor targeting

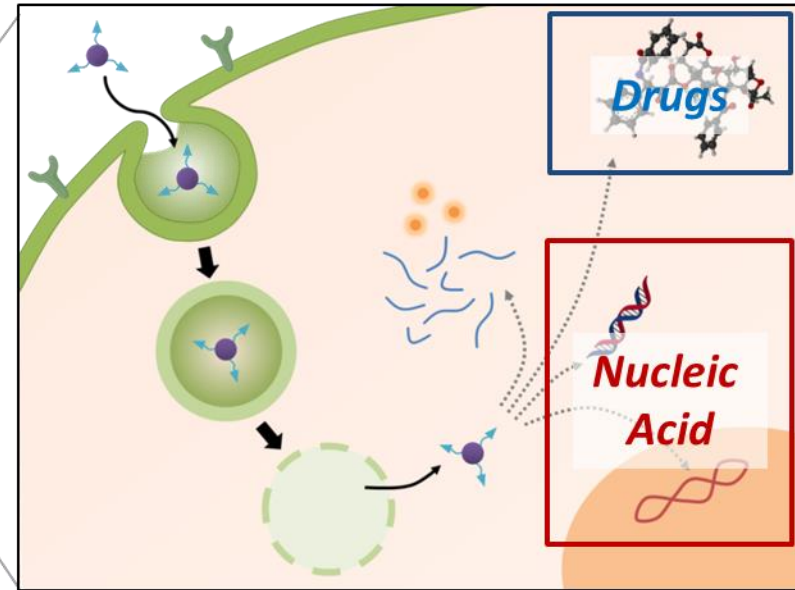
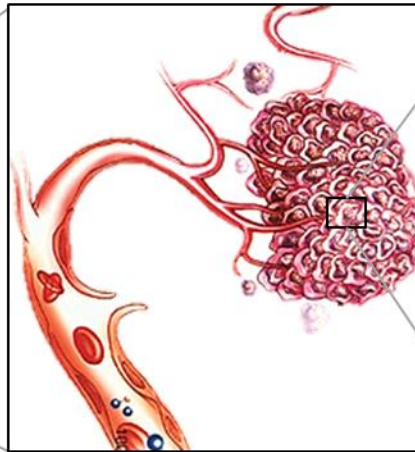
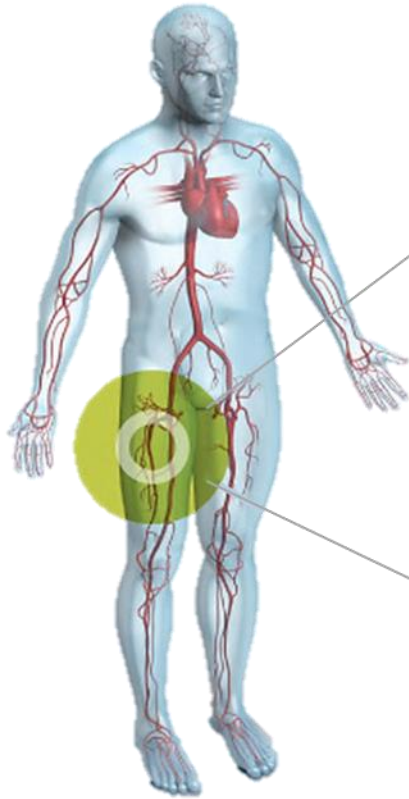
In Blood Circulation



Disease Tissue



Inside the Cells

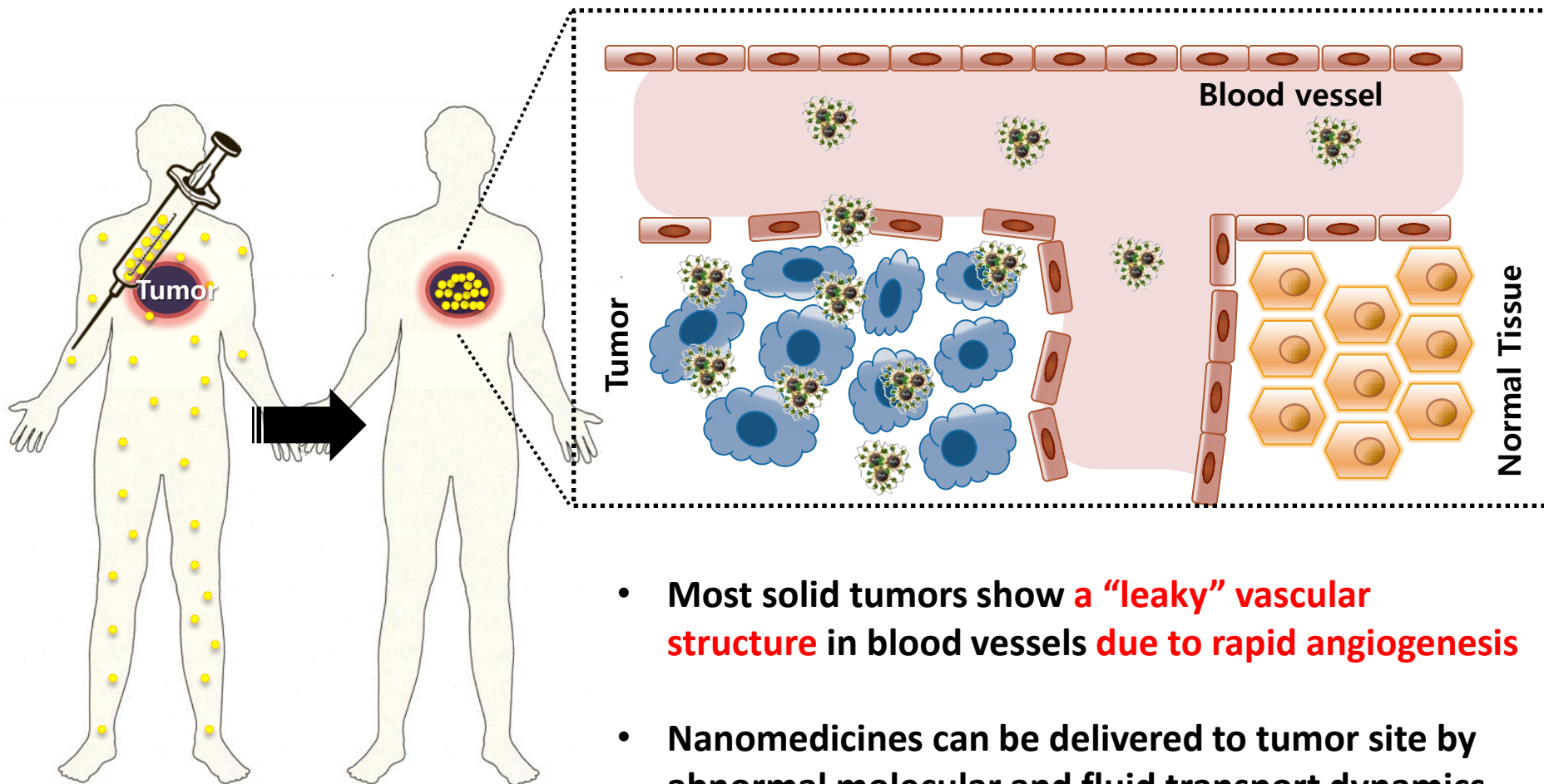


Maintain **stability**
Long circulation time

Move to a target region
EPR effect (Tumor)

Enter into the cells
Release on a desirable site
Enzyme-responsive drug release

Tumor targeting by EPR effect



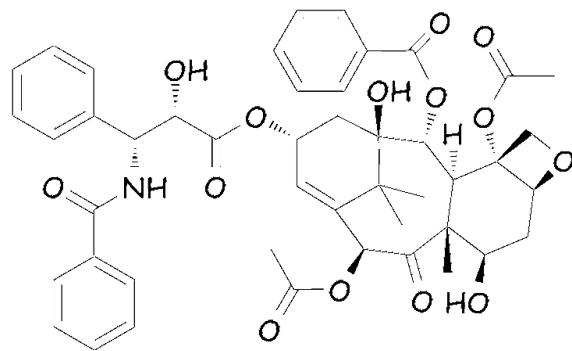
- Most solid tumors show a **“leaky” vascular structure** in blood vessels **due to rapid angiogenesis**
- Nanomedicines can be delivered to tumor site by abnormal molecular and fluid transport dynamics.

EPR : Enhanced Permeability and Retention

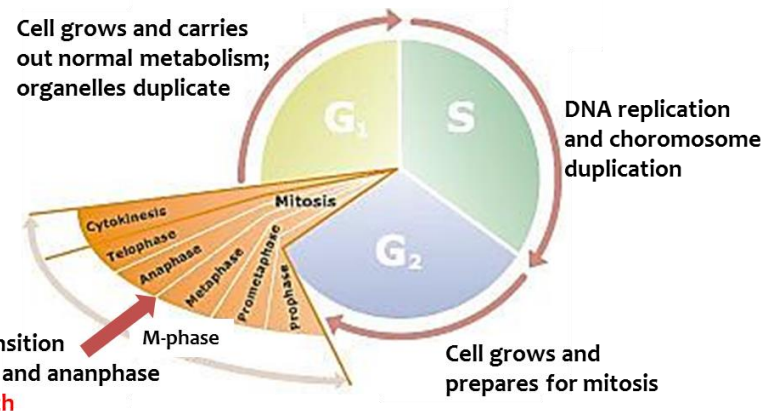
Ref: *Cancer Res.* 1986, 46, 6387-6392.

Paclitaxel (PTX) (or Taxol)

- **Natural chemotherapeutic drug**
 - Isolated from *Taxus brevifolia* (pacific yew) in 1971
- **Mitotic inhibitor**
 - Act by interfering in the normal microtubule growth during cell division
 - Arrest cell at the G₂-M phase of cell cycle
 - Induce apoptotic death
- Use in treatment of breast, ovarian, lung, head, neck cancer
- Limitation: **Poor water solubility**



Paclitaxel

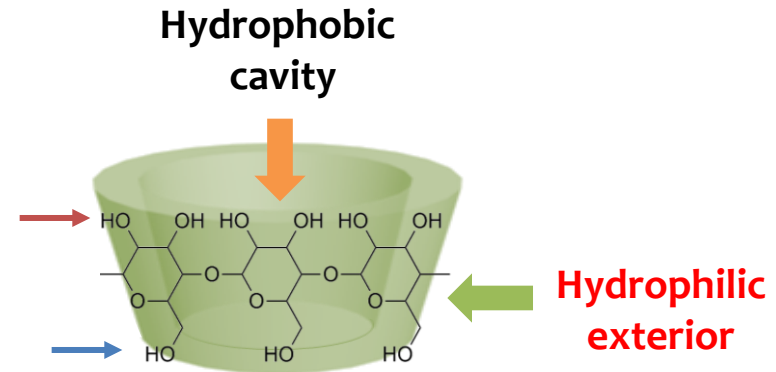
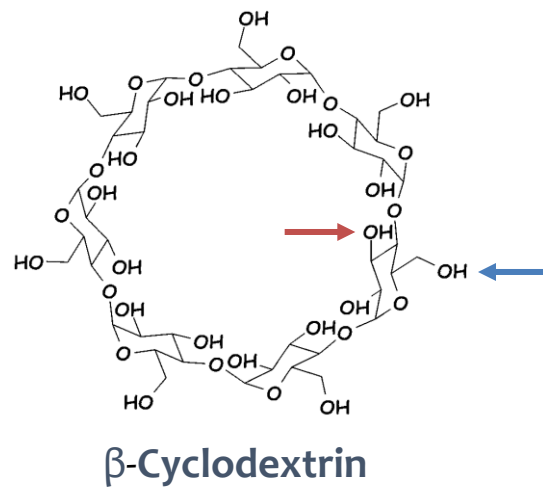


Ref: *J. Med. Chem.* **2006**, *49*, 7253-7269.

Cyclodextrin (CD)

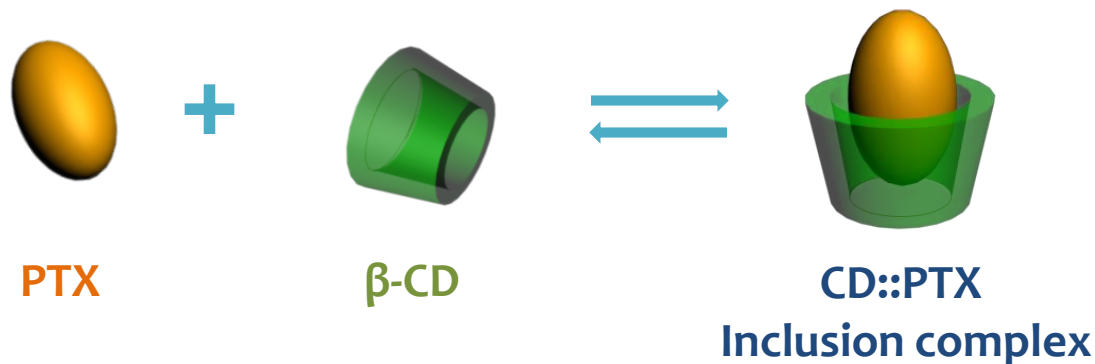
■ Cyclic oligosaccharides

- α -D-1,4-glucopyranose linked
- Produced from starch by means of enzymatic conversion



Cyclodextrin	Number of glucose ring	Height (nm)	Diameter of cavity (nm)	Diameter of exterior (nm)
α -cyclodextrin (α -CD)	6	0.79 ± 0.01	0.47-0.53	1.46 ± 0.04
β -cyclodextrin (β -CD)	7	0.79 ± 0.01	0.60-0.65	1.54 ± 0.04
γ -cyclodextrin (γ -CD)	8	0.79 ± 0.01	0.75-0.83	1.75 ± 0.04

CD-PTX inclusion complex



- **Driving force**
: Hydrophobic interaction
between the aromatic ring of PTX and the cavity of CD
- **Enhance the water-solubility of PTX**

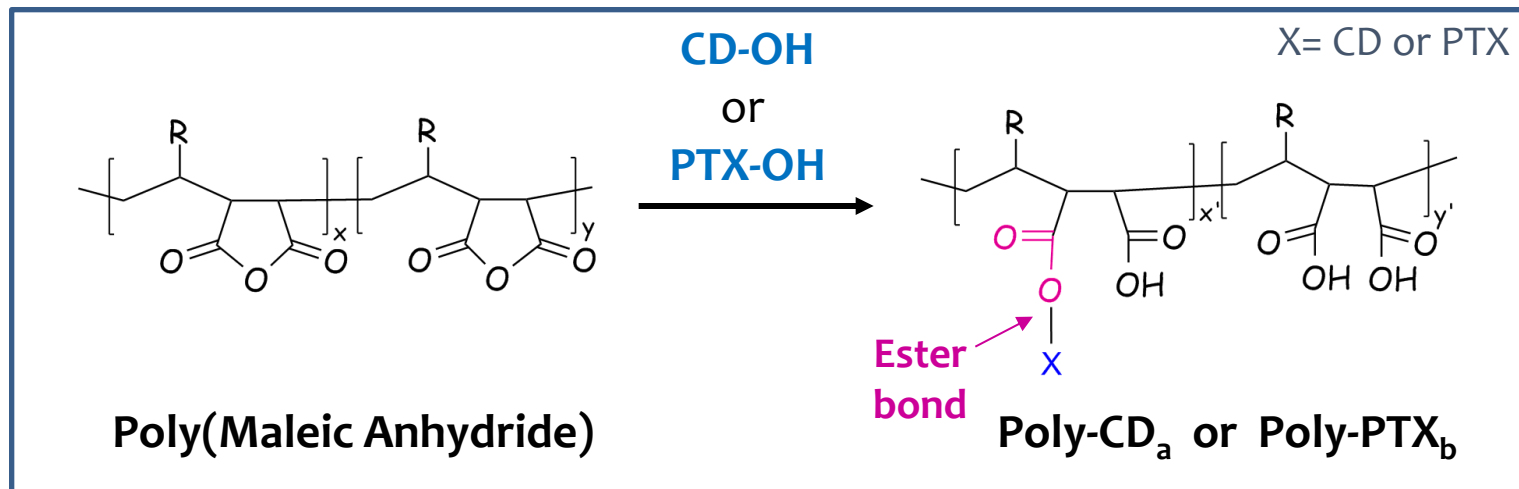
However ,
there is need further advance on it for intelligent drug delivery.

R. Namgung *et al.*, *Nat. Commun.* **2014**, *5*, 3702.

Poly(Maleic Anhydride)

Polymer for Conjugating CDs and PTXs

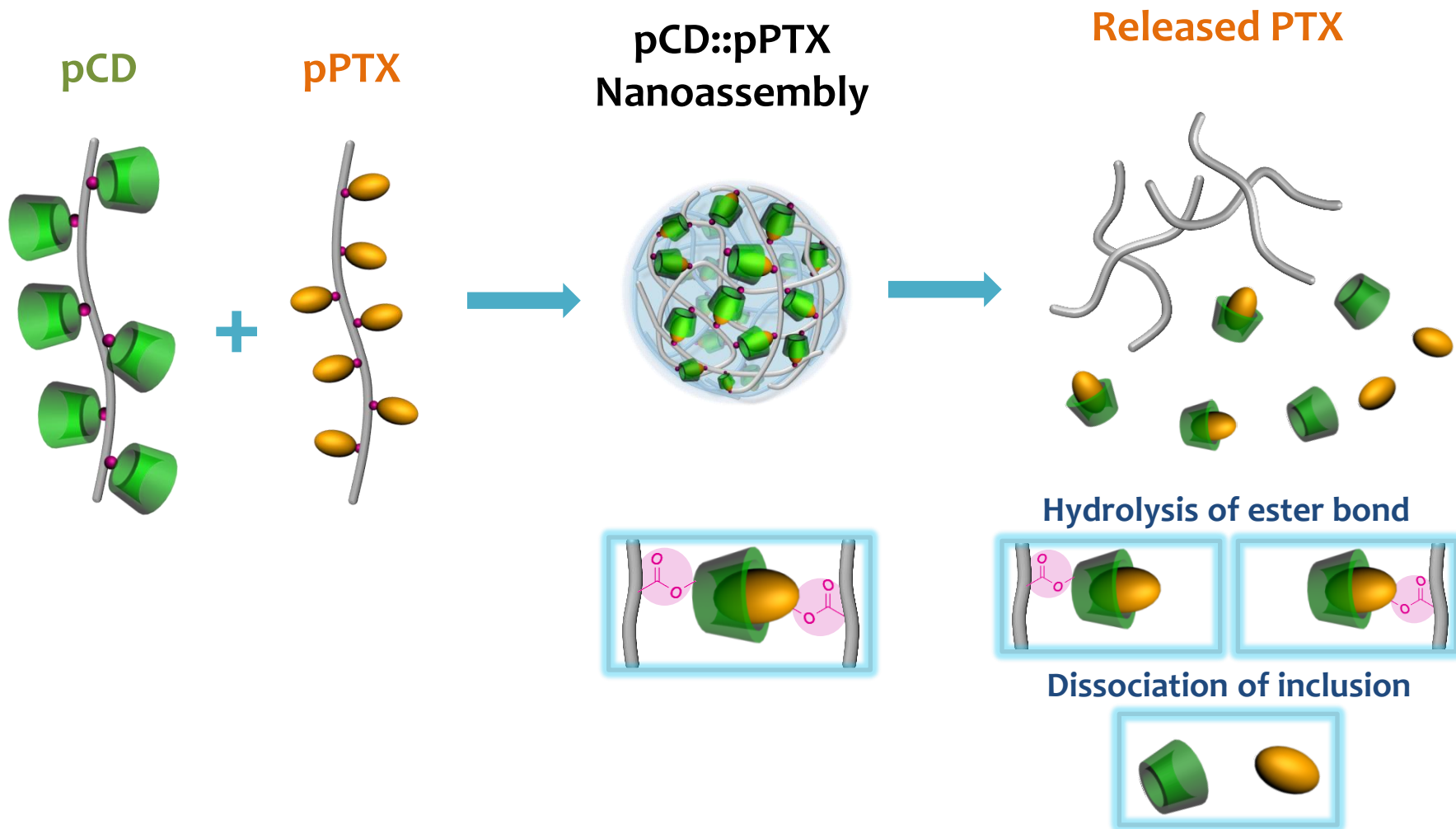
- Copolymer of maleic anhydride with another monomer
- Contain **active succinic anhydride groups** in each repeating units
- Graft **molecules having hydroxyl group** on the polymer
 - form **degradable ester bond**
 - render carboxylic acid group on polymer backbone by ring-opening



R. Namgung *et al.*, *Nat. Commun.* **2014**, *5*, 3702.

Overall scheme

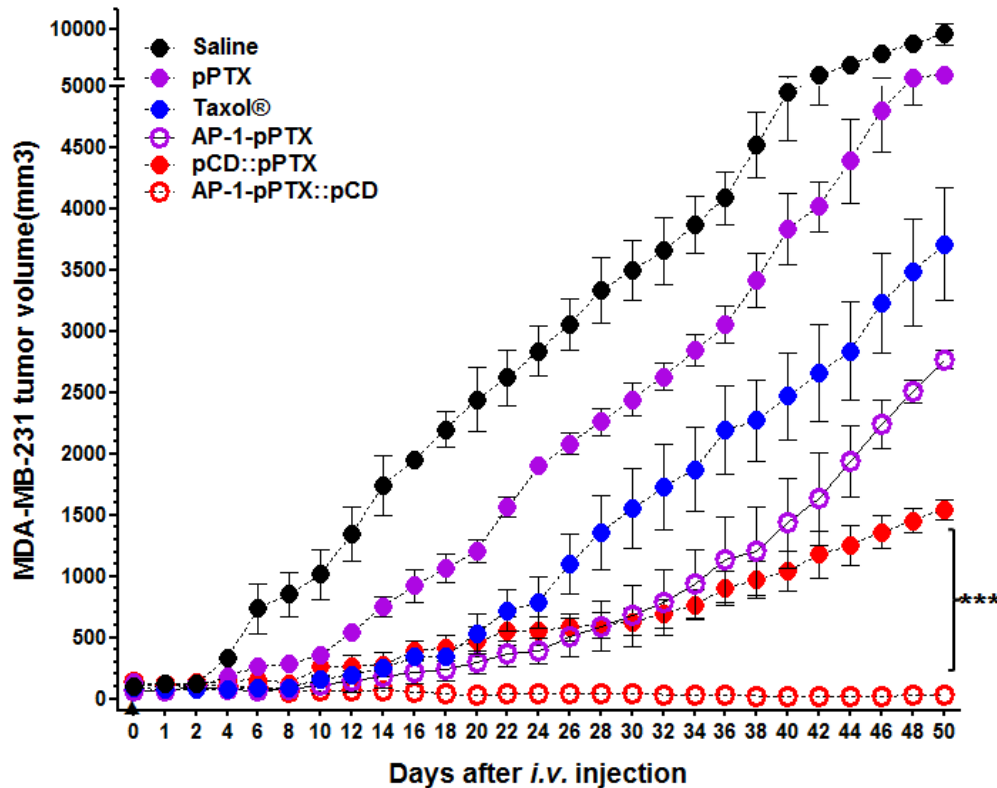
- ‘Conjugation’ plus ‘Inclusion-Complexation’



In collaboration with Prof. A. Hoffman (U of Washington)

R. Namgung *et al.*, *Nat. Commun.* **2014**, *5*, 3702.

AP-1-targeting ligand-modified carrier



pPTX micelle: fast drug release, but short-term effect

AP-1-pCD::pPTX: higher stability → sustained drug release → long-term effect



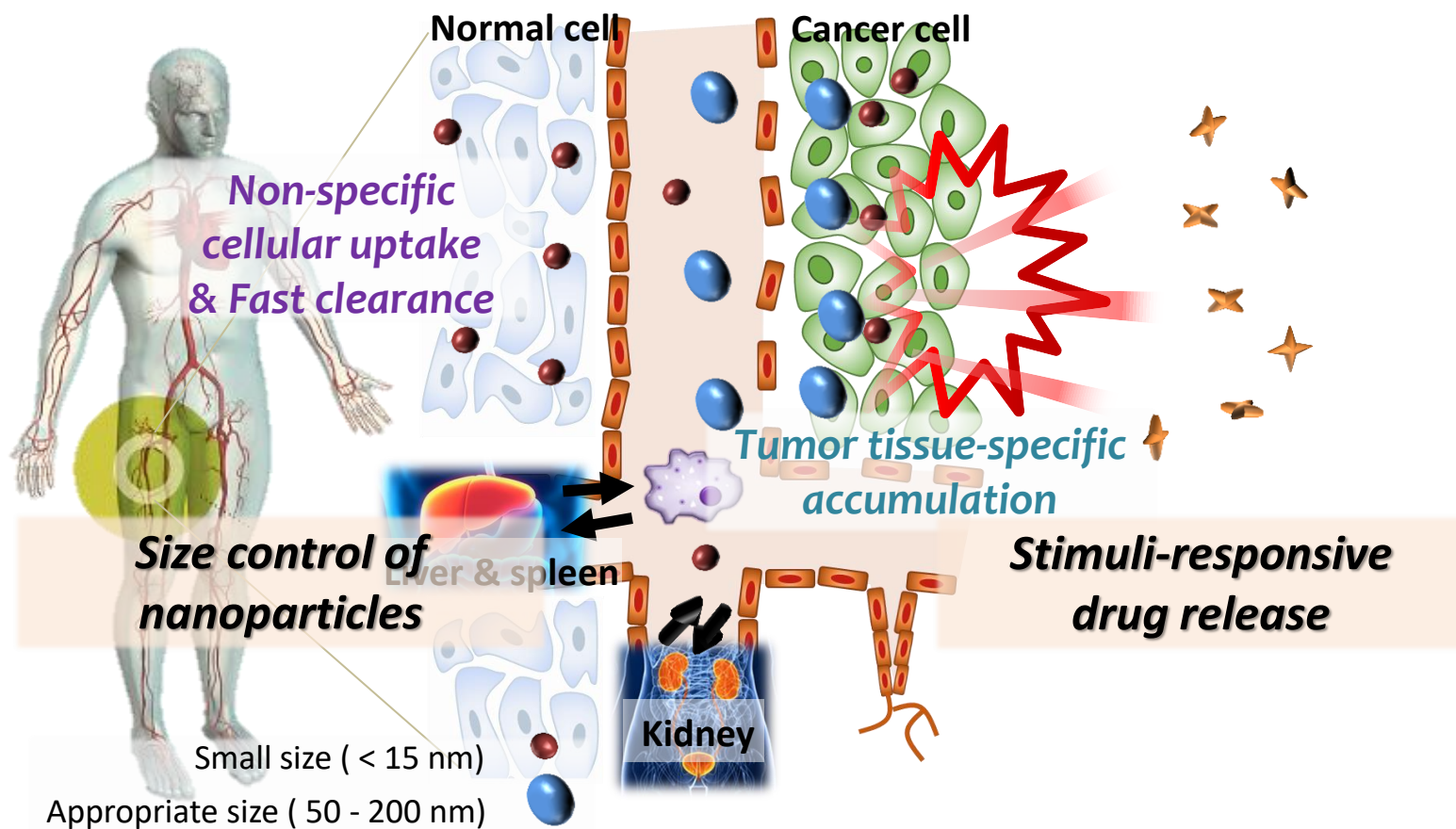
R. Namgung *et al.*, *Nat. Commun.* 2014, 5, 3702.

**Programmed nanoparticle loaded nanoparticle for
deep penetrating three-dimensional cancer therapy**

Won Jong Kim, Ph. D.

POSTECH

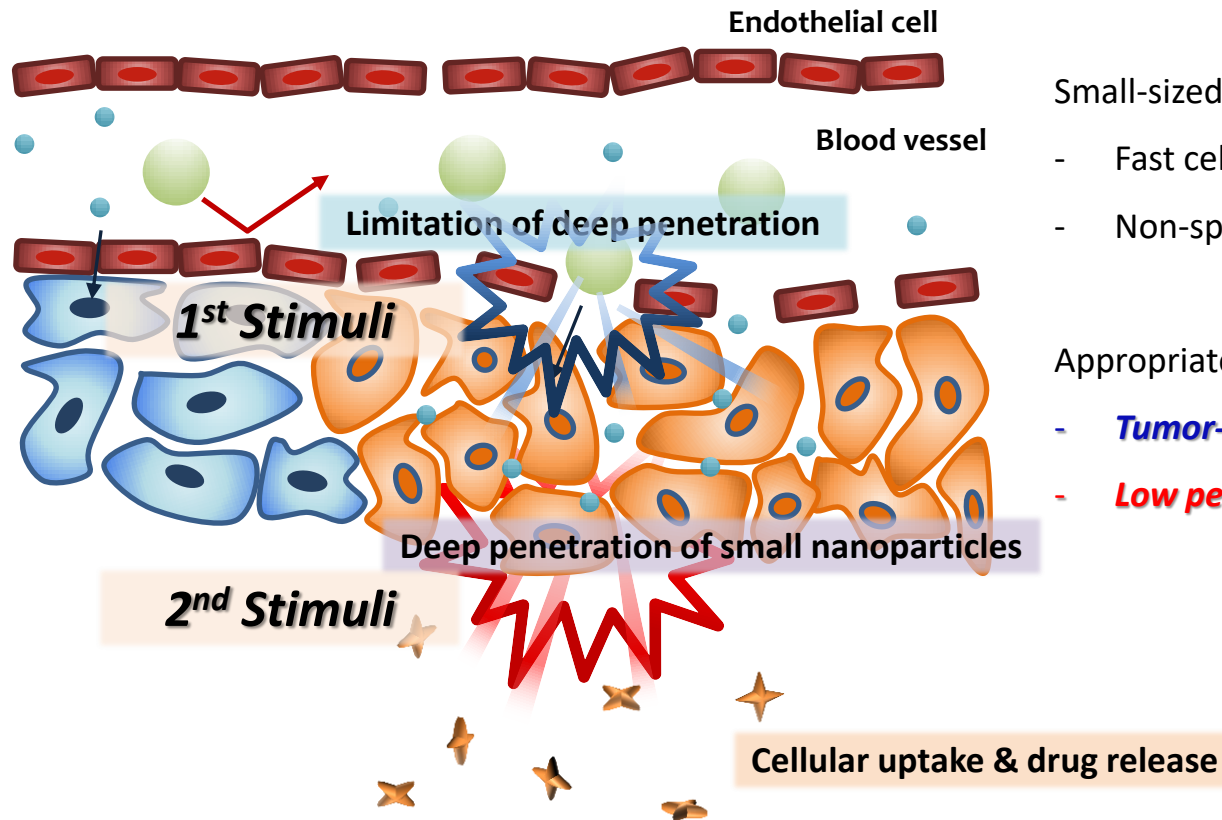
Size Dependent Biological Barriers in Drug Delivery System



Appropriate Size and Site-Specific Release for Efficient Delivery of Drugs

Ref. Clin. Cancer Res. 2012, 18, 3229-3241.
J. Kim et al. J. Control. Release 2016, in press.

Overcoming Low Penetration into Tumor Tissue



Small-sized nanoparticle (<15 nm)

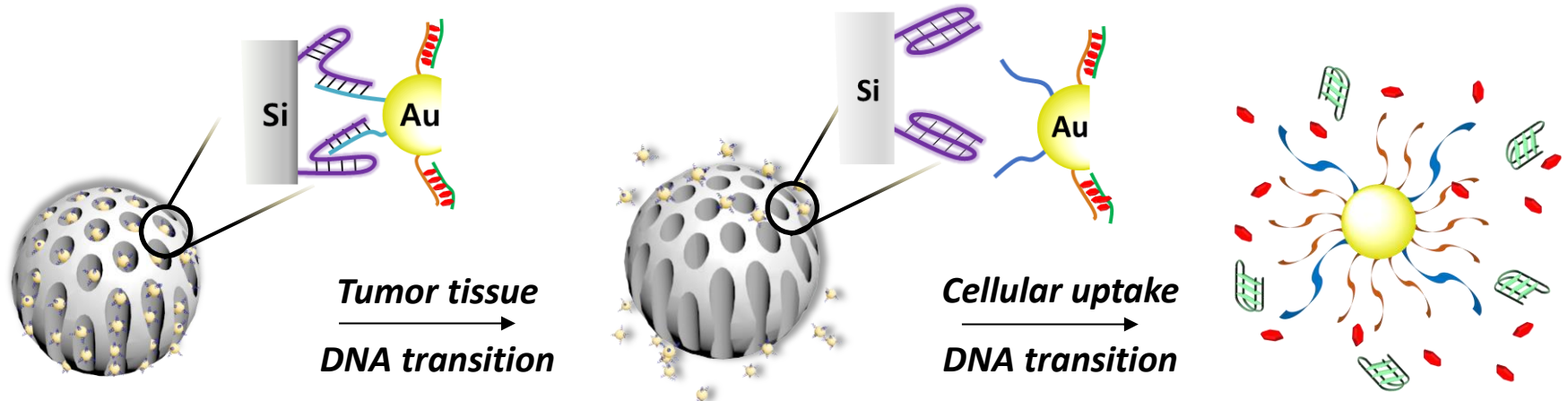
- Fast cellular uptake
- Non-specific cellular uptake

Appropriate sized nanoparticle (100-200 nm)

- **Tumor-specific uptake**
- **Low penetration ability**

Introducing the concept of **“Tumor-vector (100-200 nm)”**
to carry **bundles of small nanoparticles (<15 nm)** with drugs

Tumor-vector equipped with three-stage propulsion motioned by DNA and LPMSN



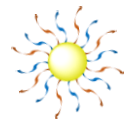
EPR Effect

Deep Penetration

Drug Release



Large pore MSNs
(LPMSNs)



DNA-AuNPs

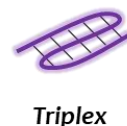
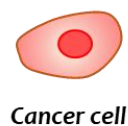
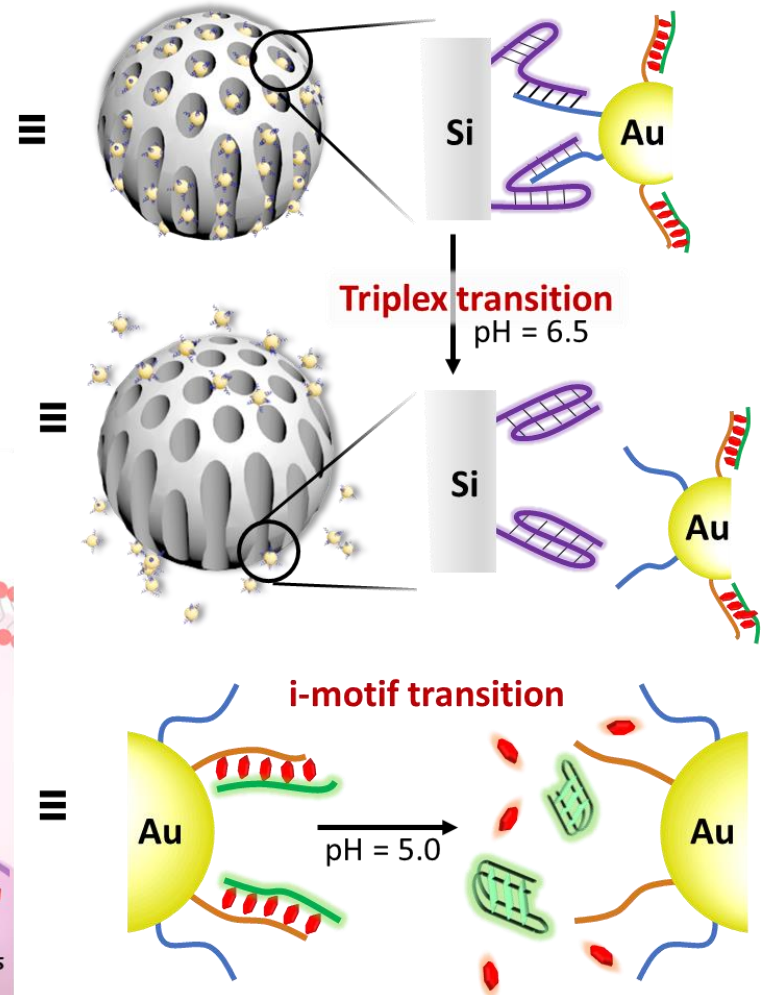
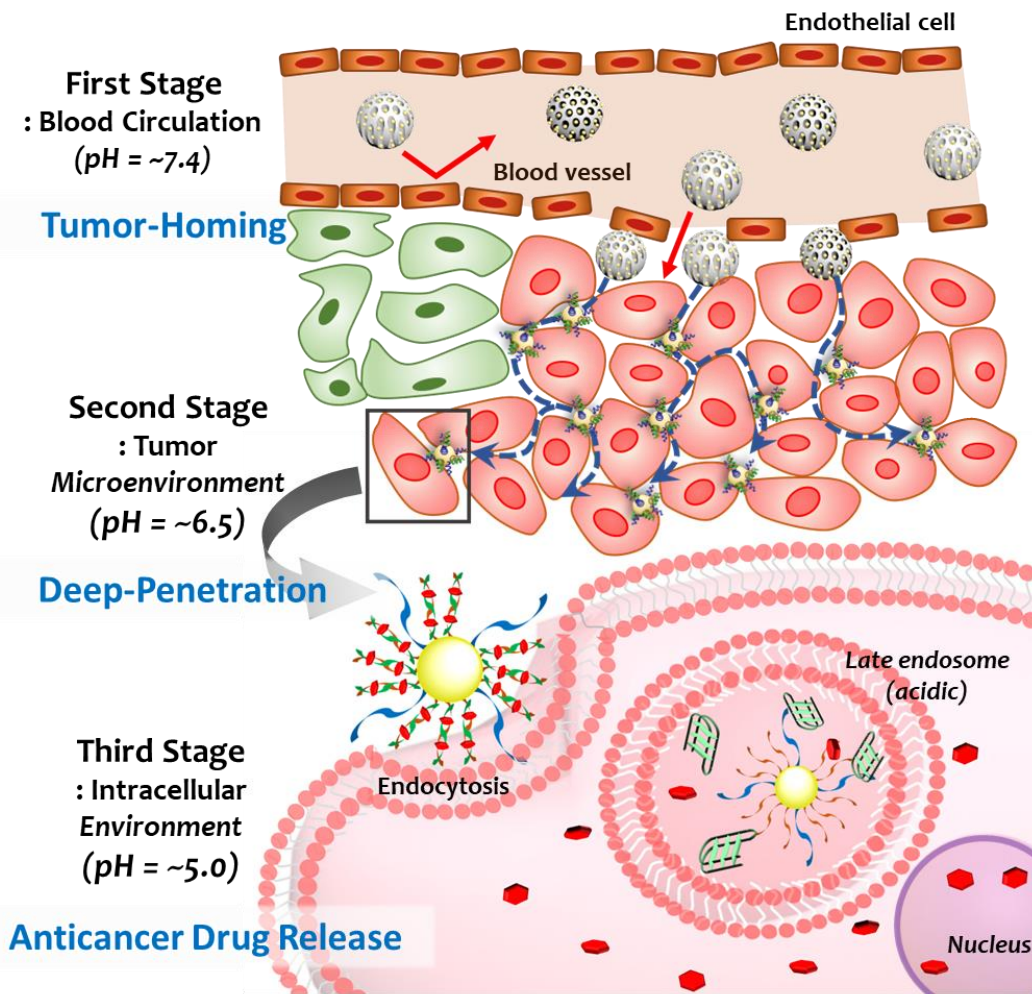


pH-responsive DNAs

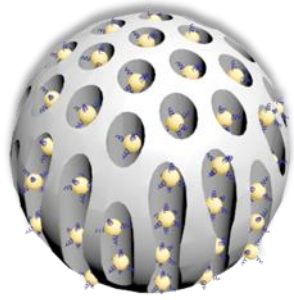


DOX

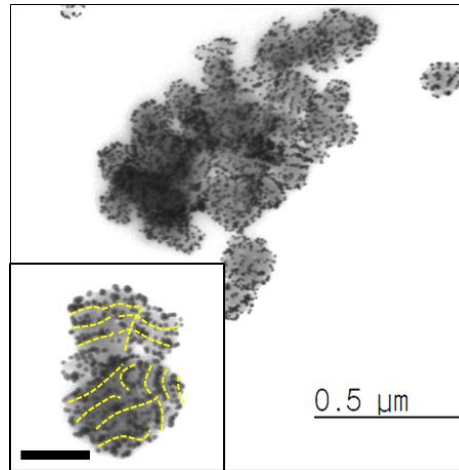
Strategy



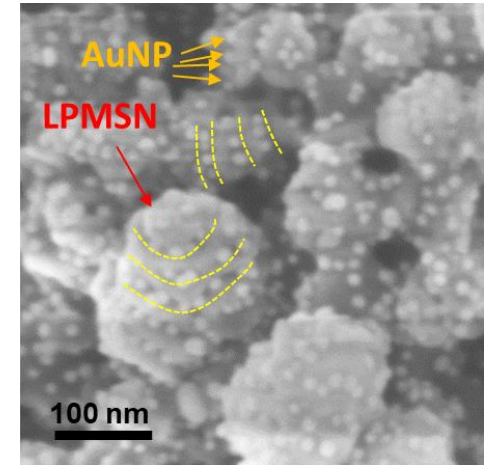
Characterization of Tumor-vector



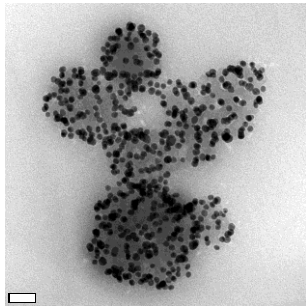
Tumor-vector
(*via* DNA hybridization)



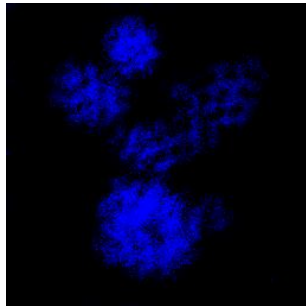
TEM image



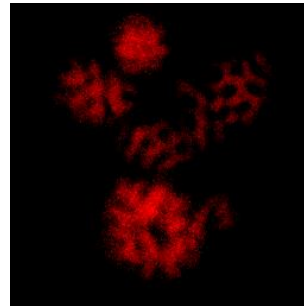
SEM image



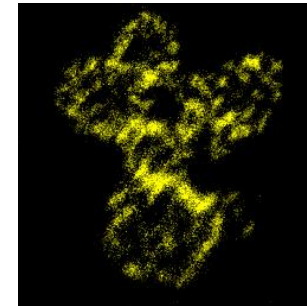
BF



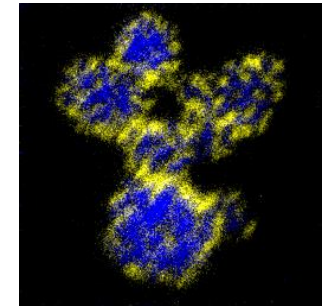
Si map



O map



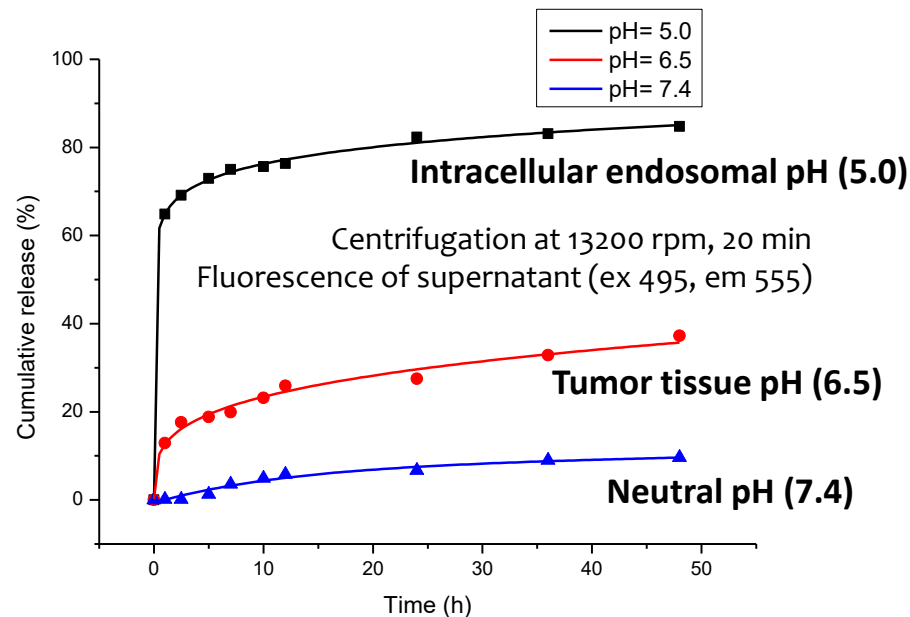
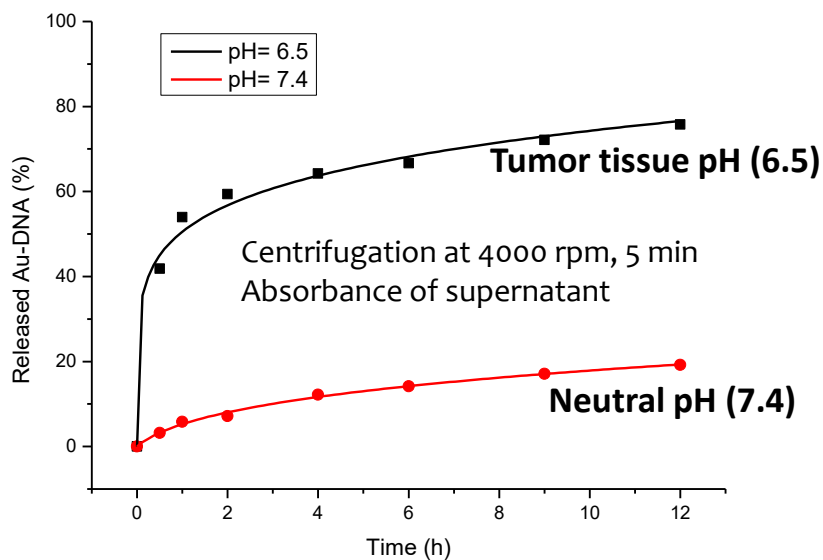
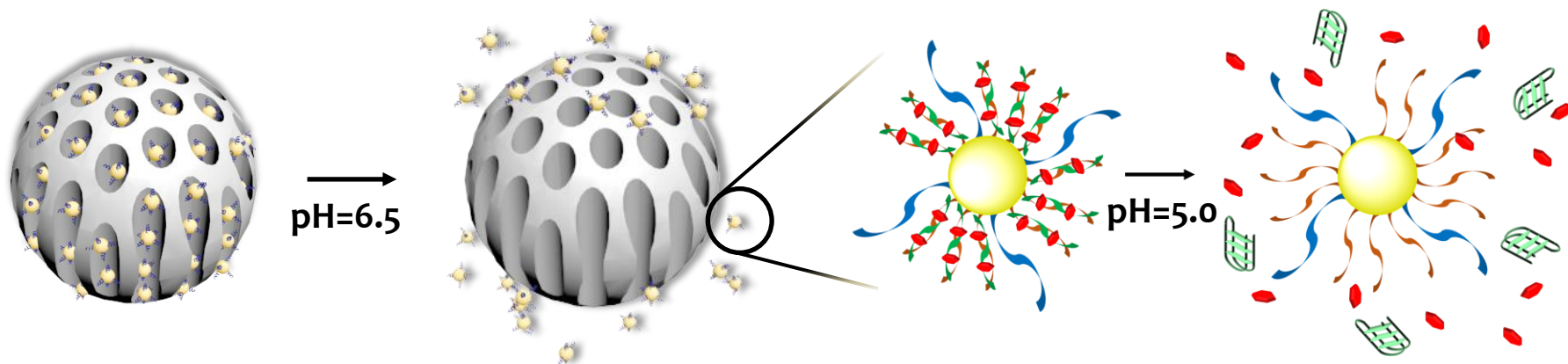
Au map



Si + Au map

Successful formation of tumor-vector via DNA hybridization

pH-Responsive AuNP and Drug Release



Au release at tumor microenvironmental pH

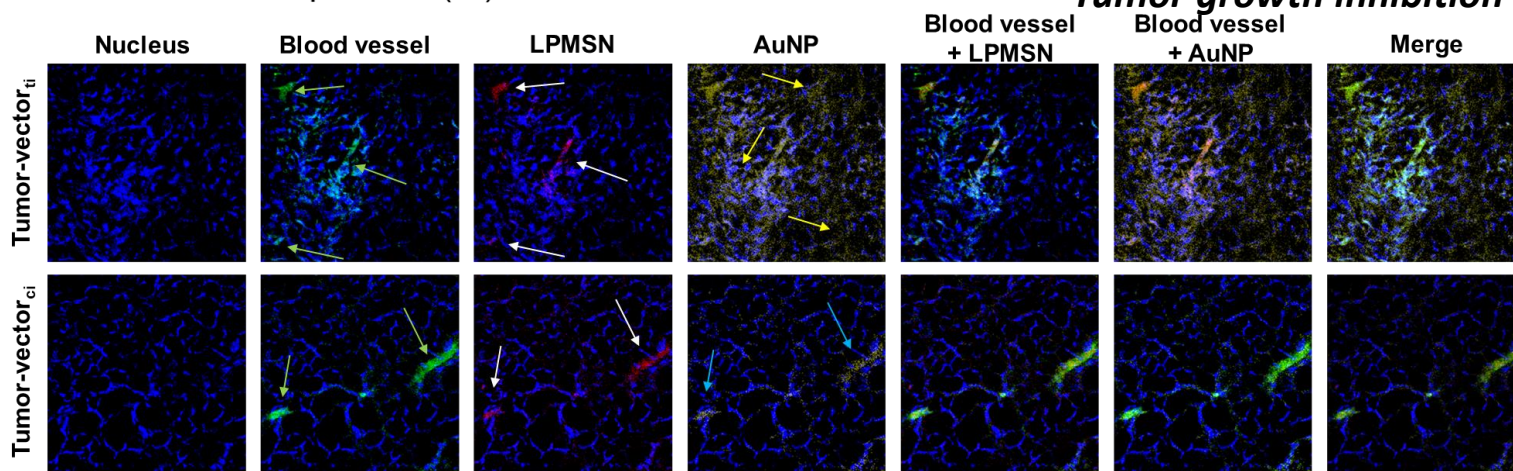
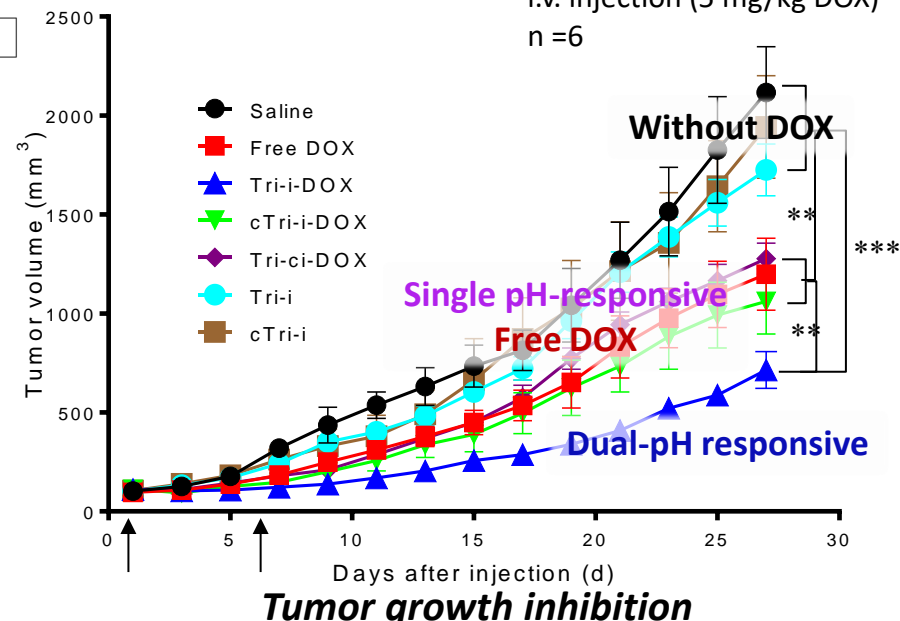
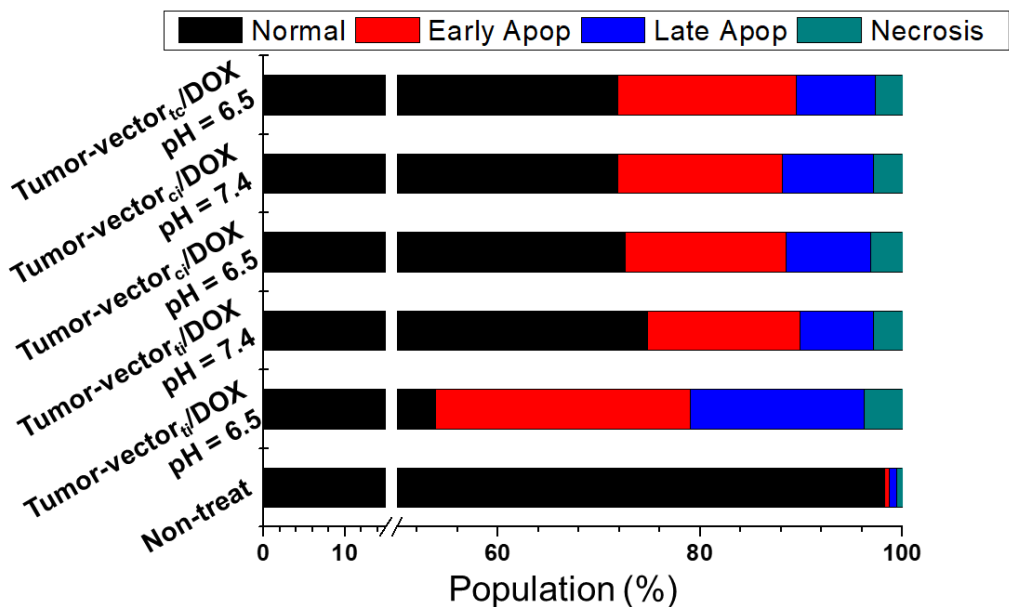
DOX release intracellular endosomal pH

The amount of loaded AuNPs and DOX was calculated by back-titration of the supernatant
 Approximately 49 AuNPs in 1 MSN (in TEM image)

J. Kim *et al.* *Advanced Materials*, in press.

In Vivo Antitumor Effect and Deep Penetration

MDA-MB-231 cell xenograft
i.v. injection (5 mg/kg DOX)
n = 6



The highest anticancer effect (EPR, deep penetration, pH-sensitive drug release)

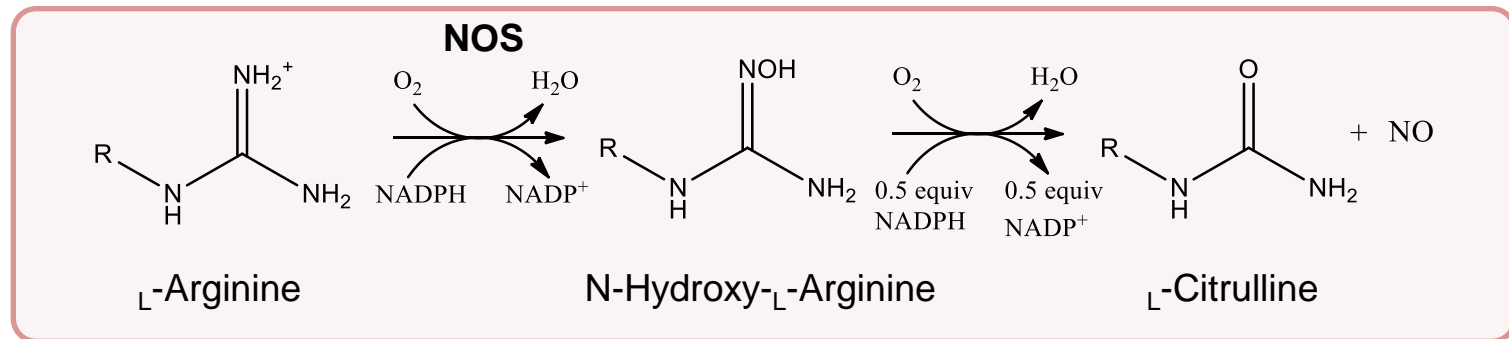
“Nitric Oxide Delivery System”

- ◆ Stimuli-responsive *NO delivery system*
- ◆ *NO-responsive* transforming hydrogel for biomedical applications

- NO

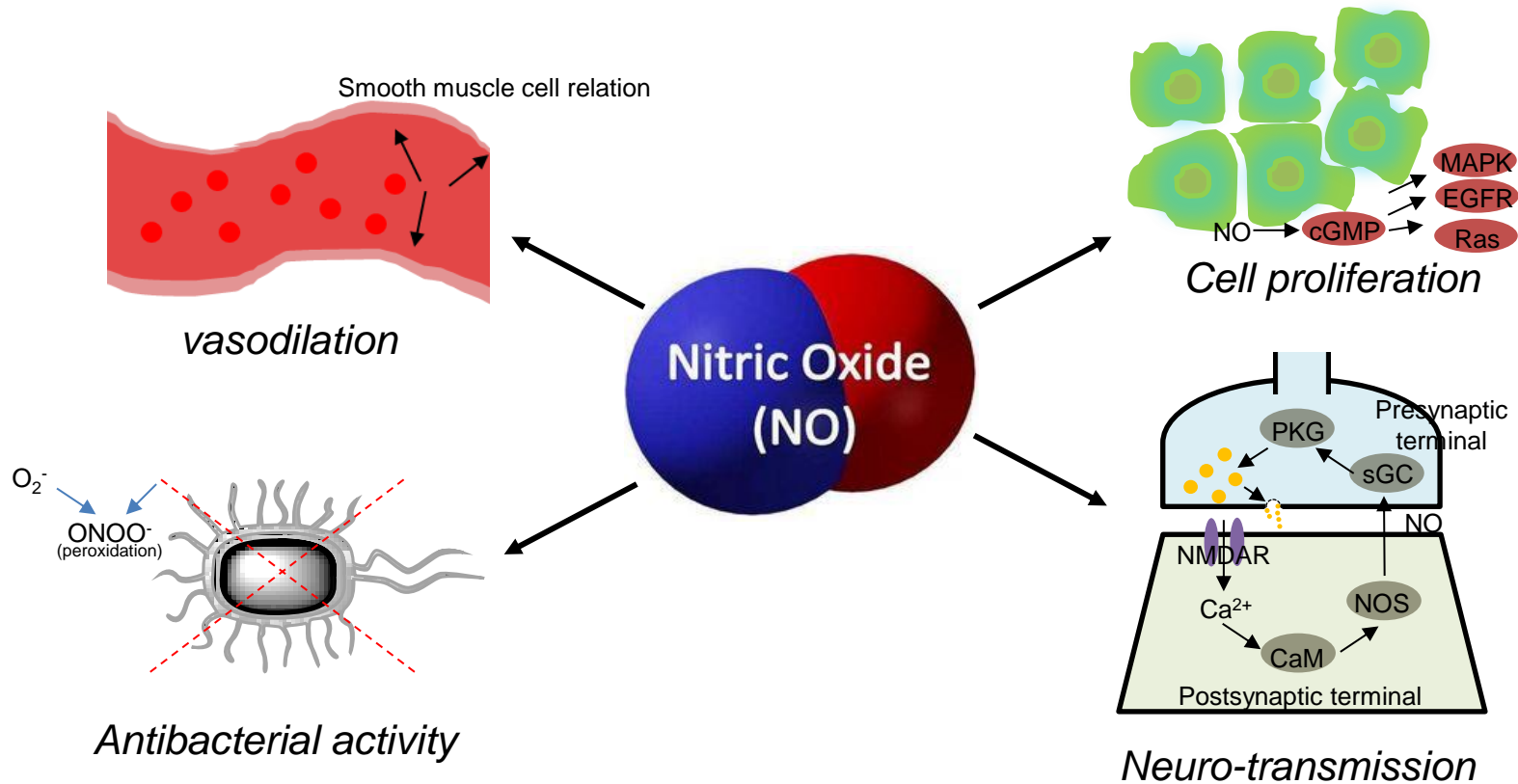
Highly reactive free radical molecule ($t_{1/2} < 6s$)
Produced by a NOS (Nitric Oxide Synthase) *in vivo*.

- In vivo* production of NO via L-Arginine



Ref. Clin. Science **2000**, 98, 507-520.
J. Kim. *et al.*, *J. Mater. Chem. B* **2014**, 2, 341-356.

Biological Function of Nitric Oxide (NO)

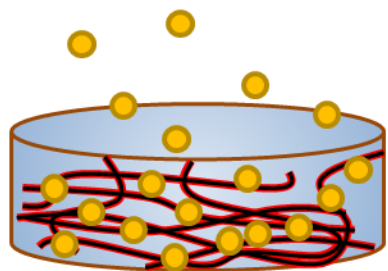


NO is pathophysiological modulator of vasodilation, cell proliferation, antibacterial activity, neurotransmission

Ref. *Cell* 1994, 79, 915-918.

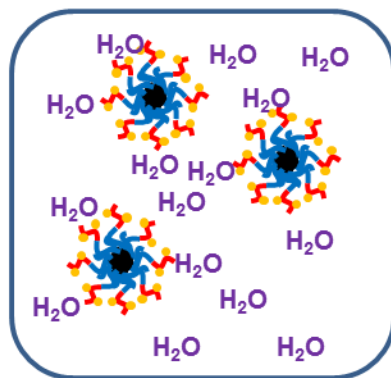
Platforms for Local NO delivery

1. NO-releasing hydrogels



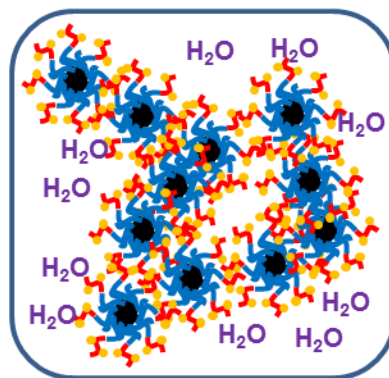
NO-delivery hydrogel

Fast NO release



Sol

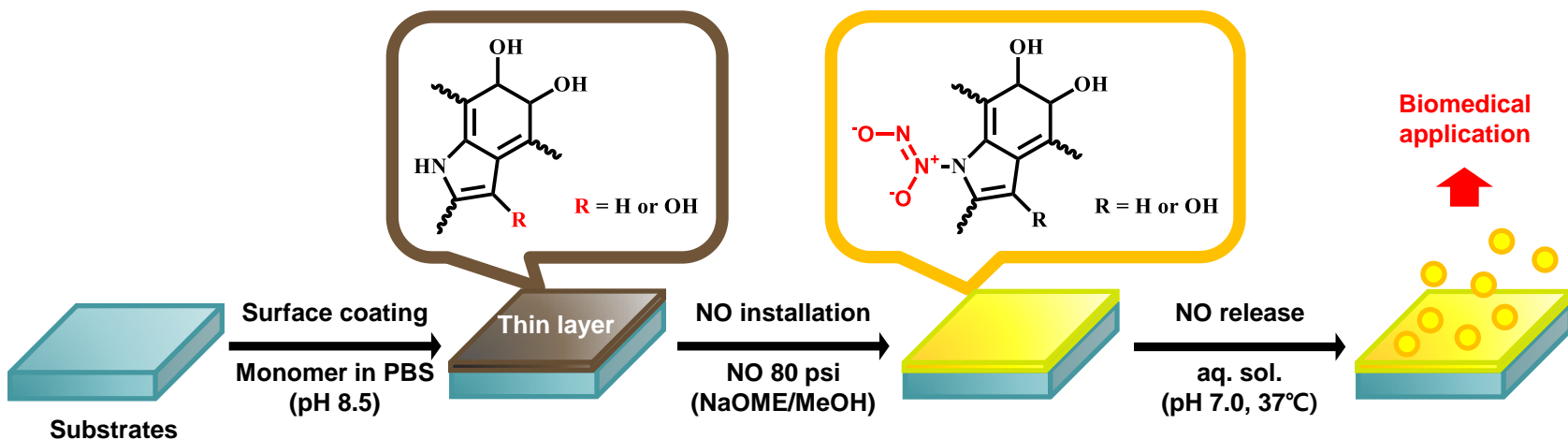
Slow NO release



Gel → Easy coating

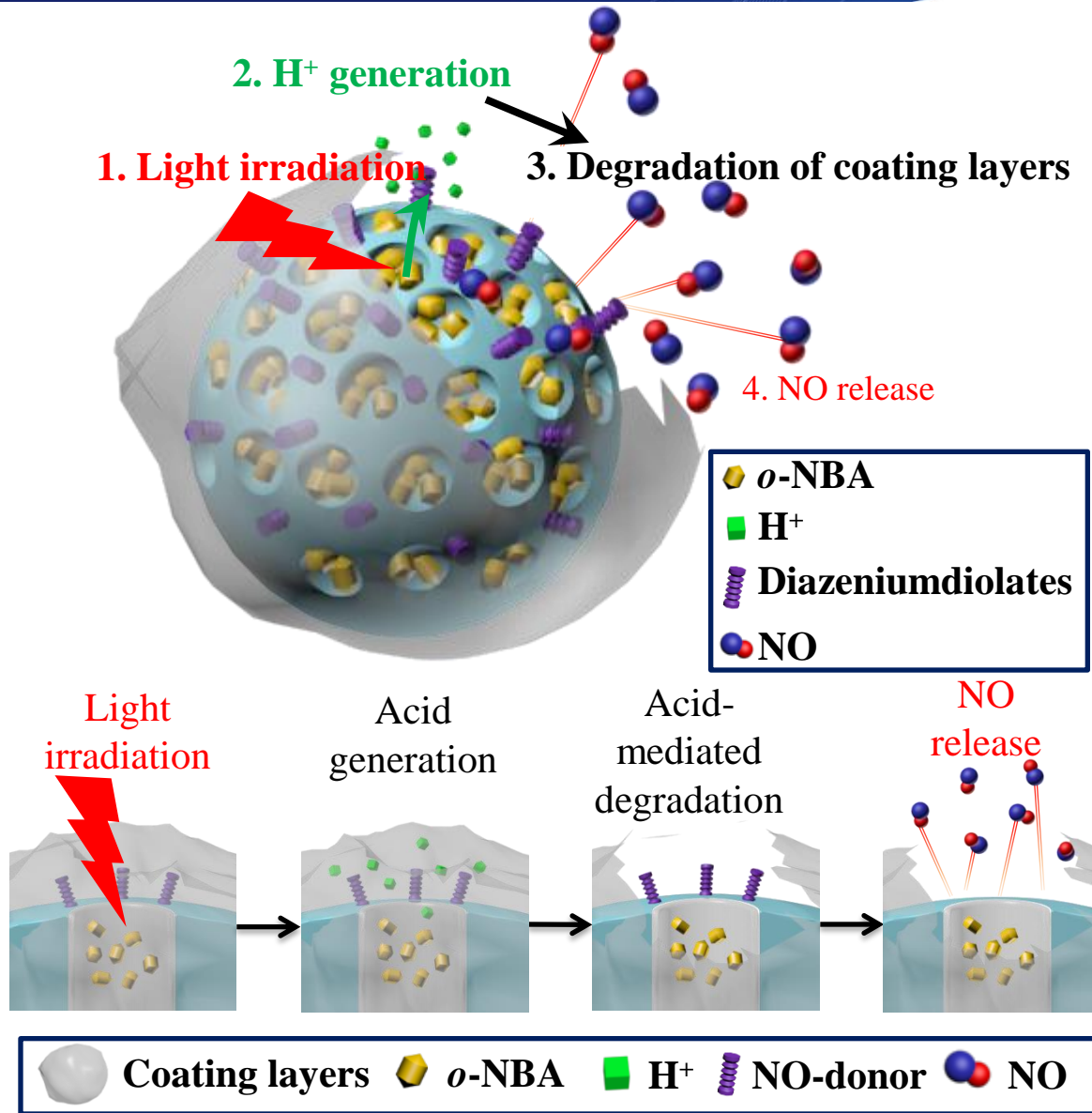
Kim, J. et al., *Bioconjug. Chem.* **2011**, *22*, 1031-1038.

2. Localized NO delivery

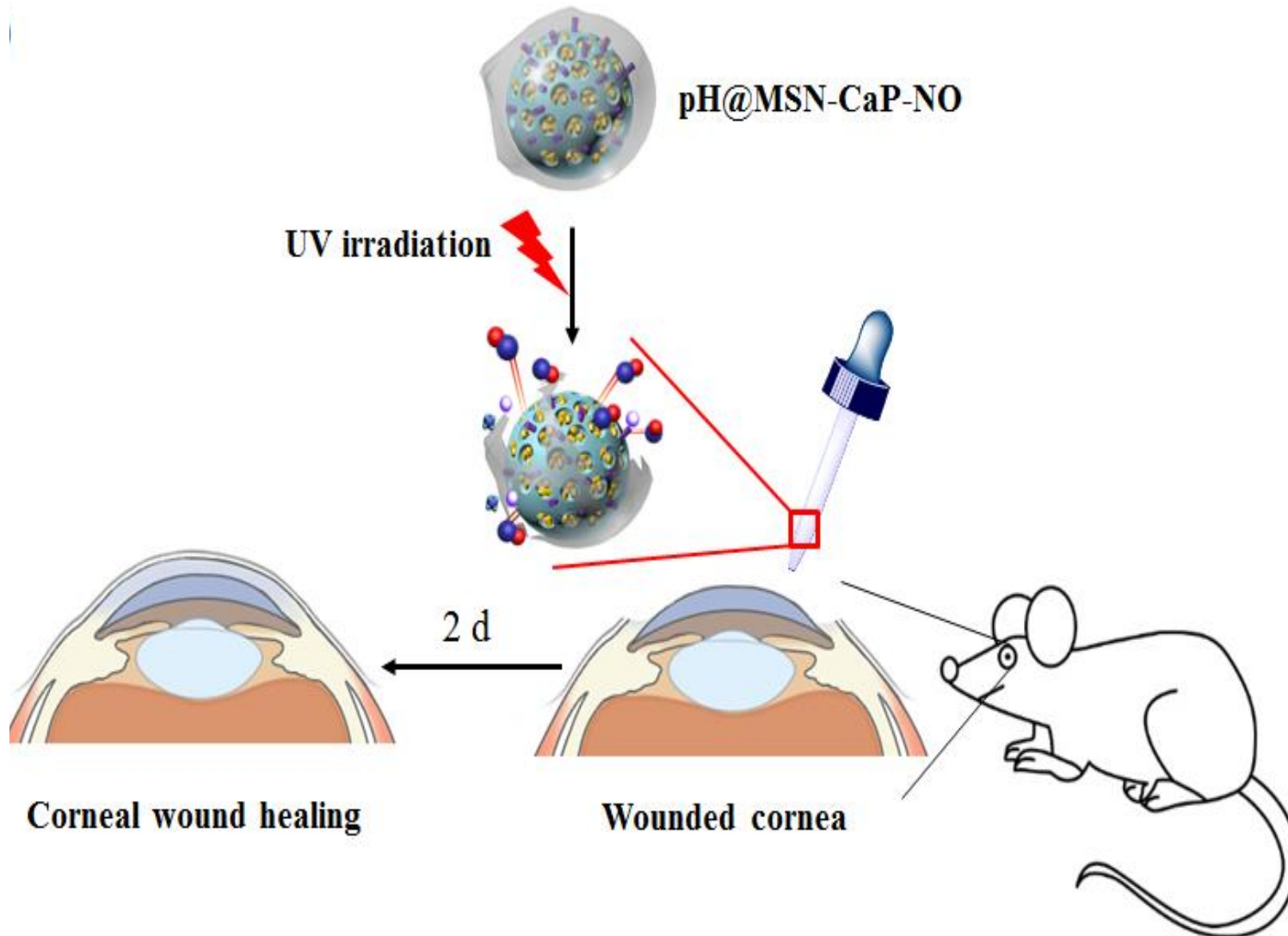


Kim, J. et al., *Angew Chem Int Ed.* **2013**, *52*, 9187-9191.

Light-triggered NO delivery system



Corneal wound healing effects

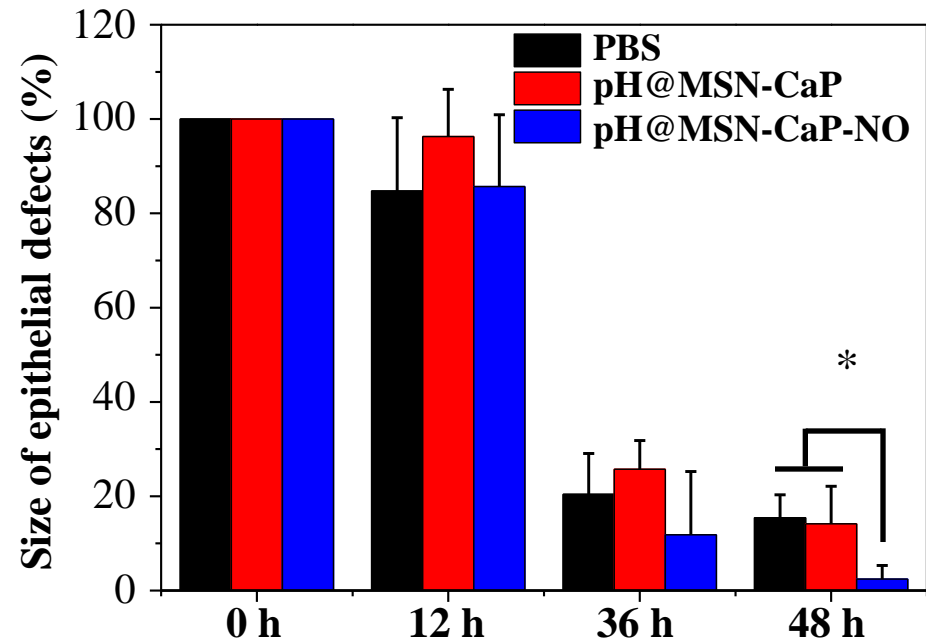
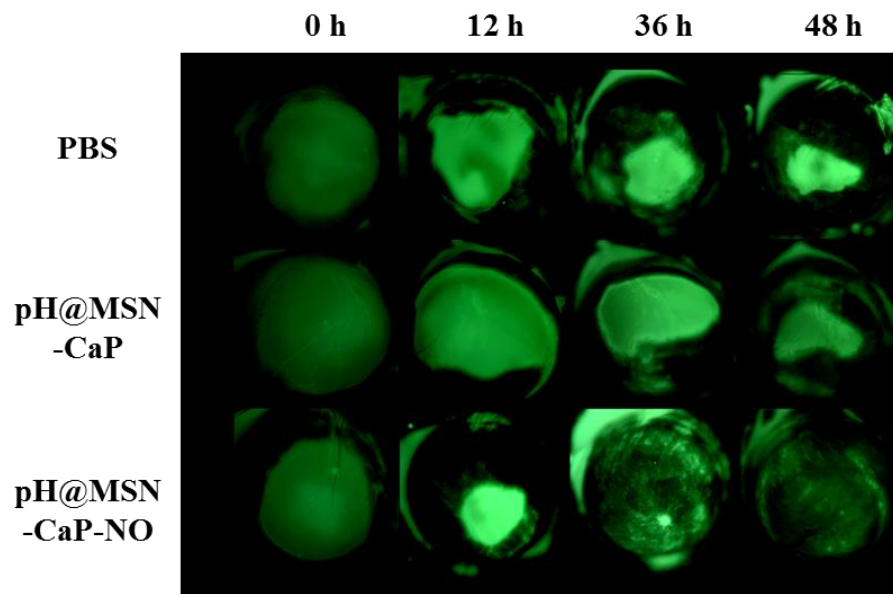


Corneal wound healing effects

Collaboration with Kim, J. H. group in SNU.

C57BL/6 mice

Wound model: created by scraping corneal epithelium with a surgical blade
5 μ L of 1mg/mL, 10 min light exposure, 4 times a day
Detection: 0.5% fluorescein sodium solution

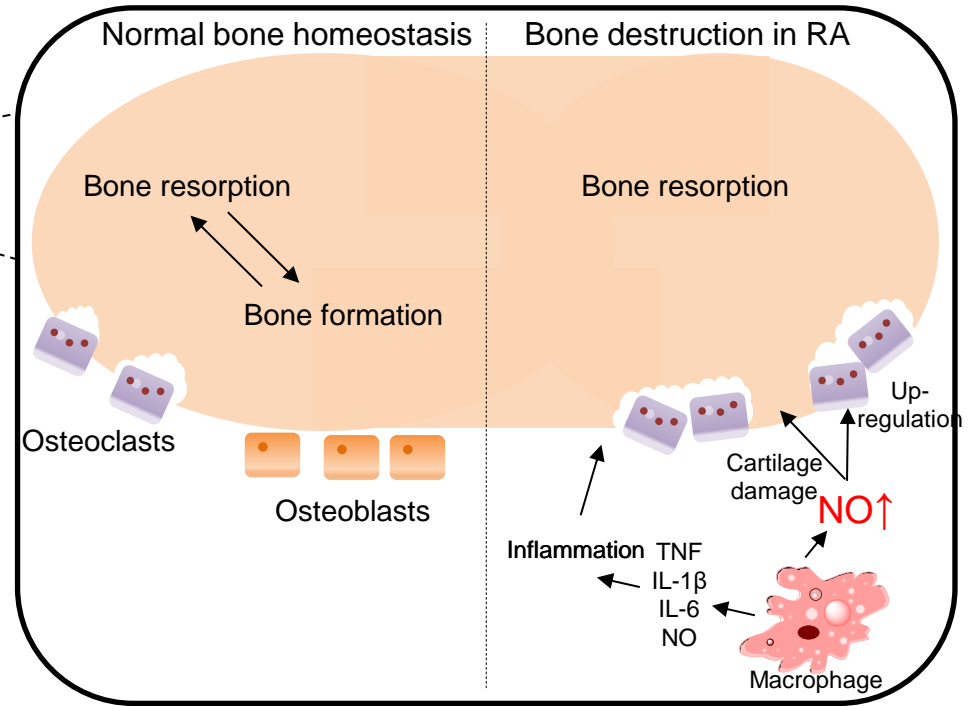


Accelerated wound healing effects on the cornea at day 2 compared to that of other control groups

Kim, J. et al. ACS Nano, 2016

Nitric Oxide in Rheumatoid Arthritis

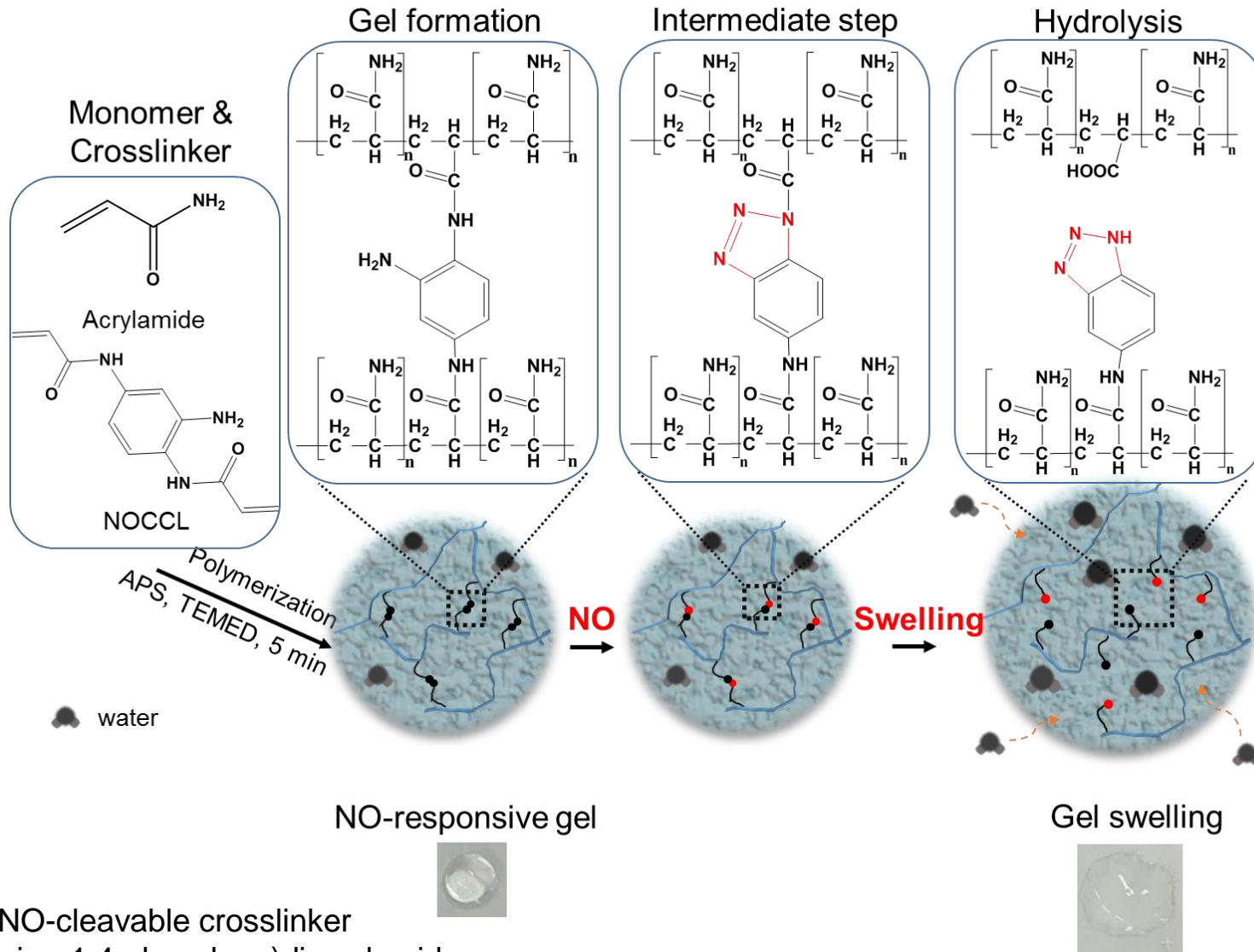
Rheumatoid Arthritis



- *NO may affect the pathogenesis of rheumatoid arthritis*
- ***NO-responsive materials*** can target cell and eliminate over-expressed NO

Ref. Nat. Rev. Rheumatol. 2009, 5, 543-548.

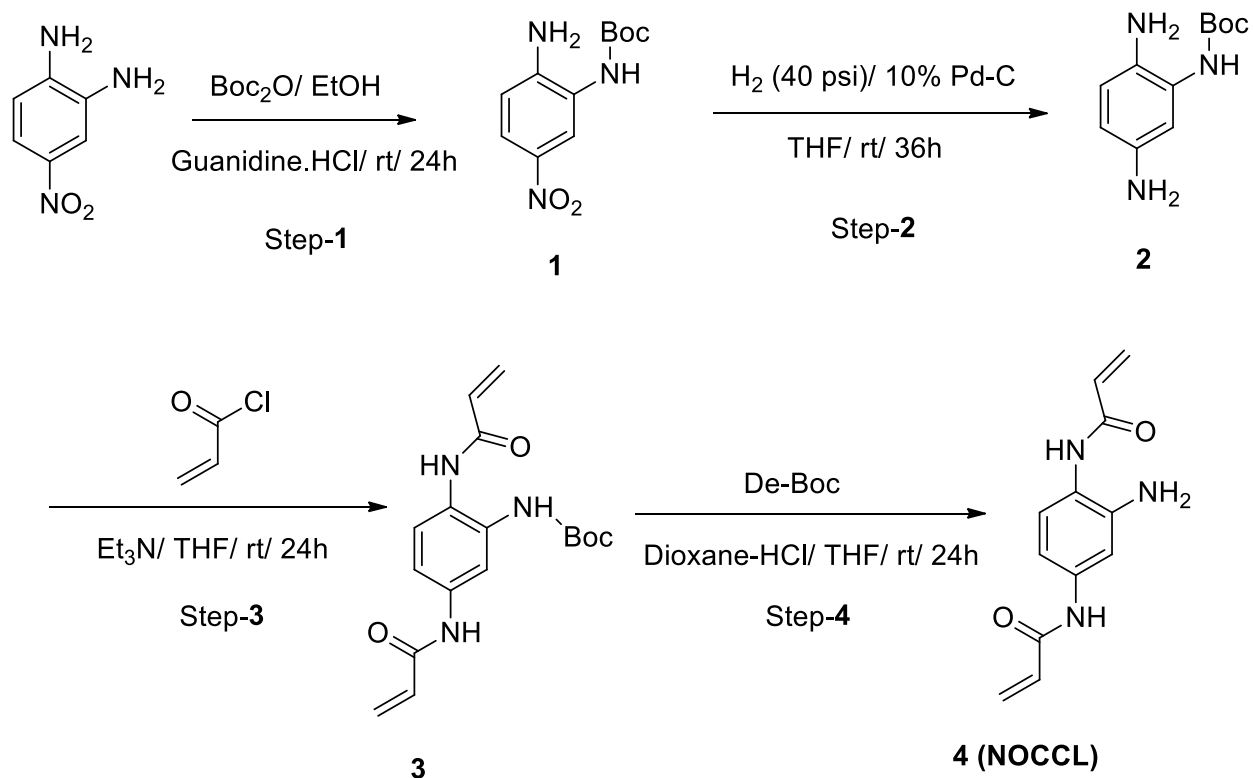
NO-Responsive Hydrogel



NOCCL : NO-cleavable crosslinker
N,N'-(2-amino-1,4-phenylene)diacrylamide

J. Park *et al.* *Advanced Materials*, in press.

Synthesis of NOCCL

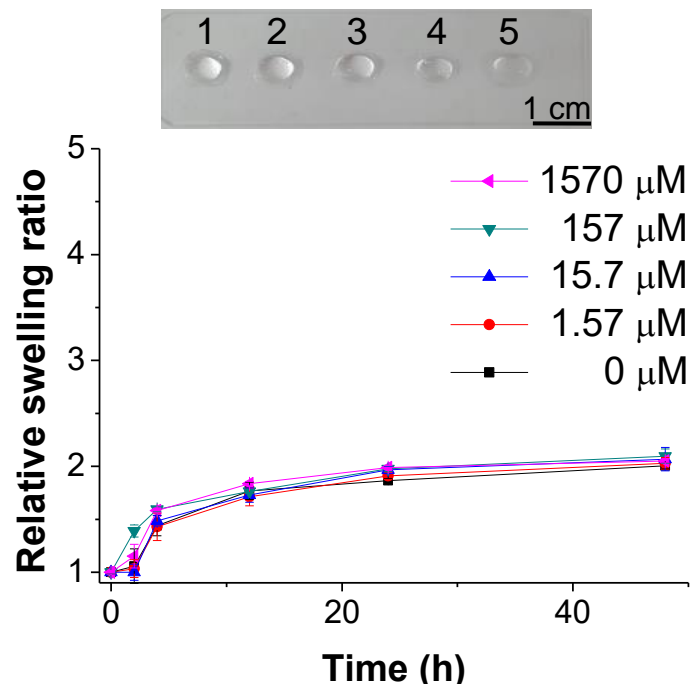
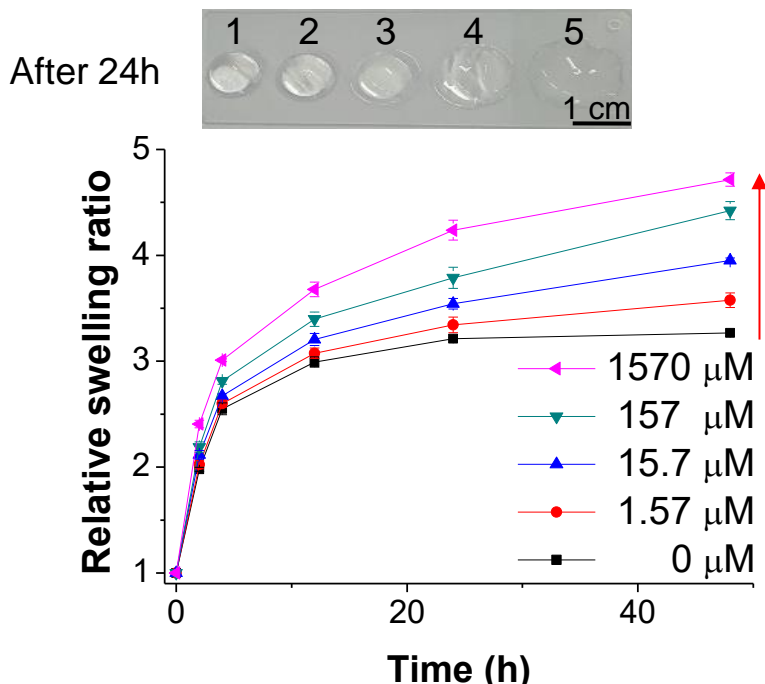
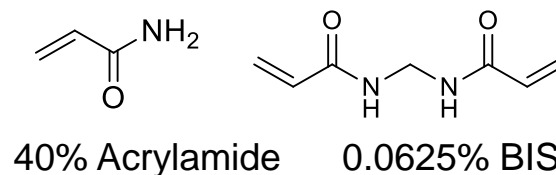
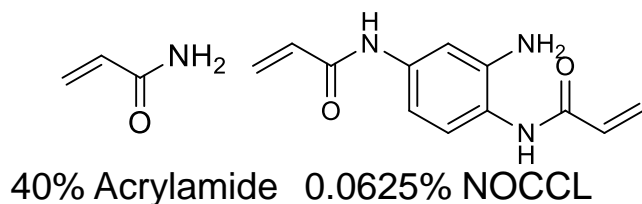


NOCCL was successfully synthesized and confirmed by ^1H NMR

J. Park *et al.* *Advanced Materials*, in press.

Swelling Test in NO Solution

N,N'methylenebisacrylamide (BIS)

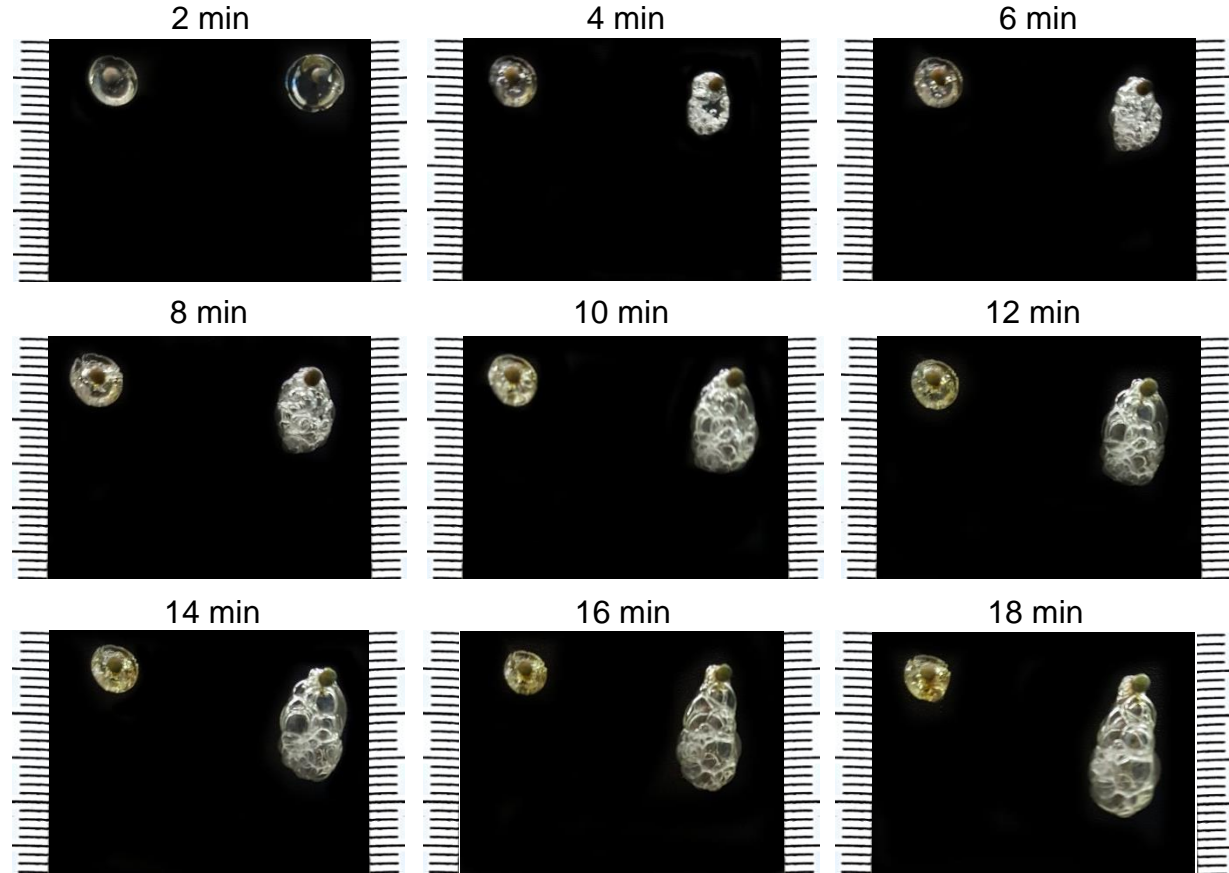
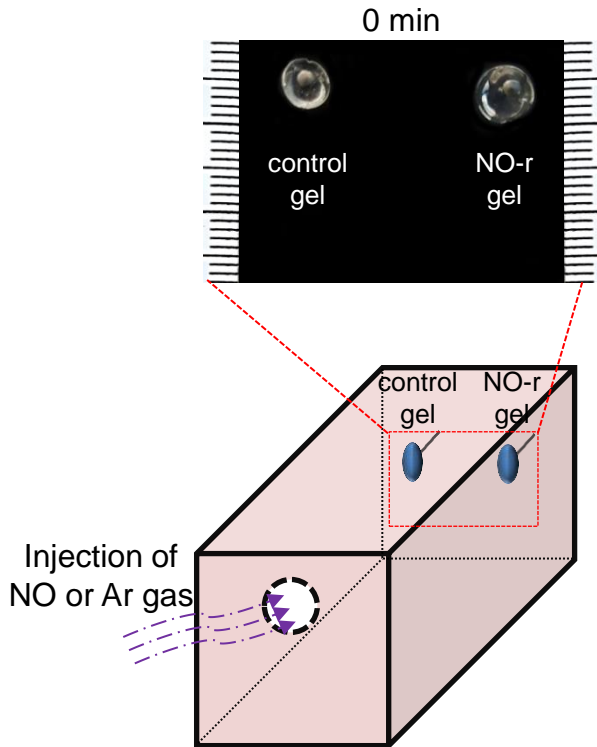


NO-responsive hydrogel swelled gradually **by the cleavage of NOCCL**

J. Park *et al.* *Advanced Materials*, in press.

Hydrogel Deformation by NO Gas

Commercial plastic bucket (10cm x 13cm x 5 cm)
0.4 cm holes that allowed gas to enter and exit



NO-responsive hydrogel deformed by NO gas

J. Park *et al.* *Advanced Materials*, in press.

Hydrogel Deformation by NO Gas

Commercial plastic bucket (10cm x 13cm x 5 cm)
0.4 cm holes that allowed gas to enter and exit

A movie demonstrating the deformation of NO-responsive gel

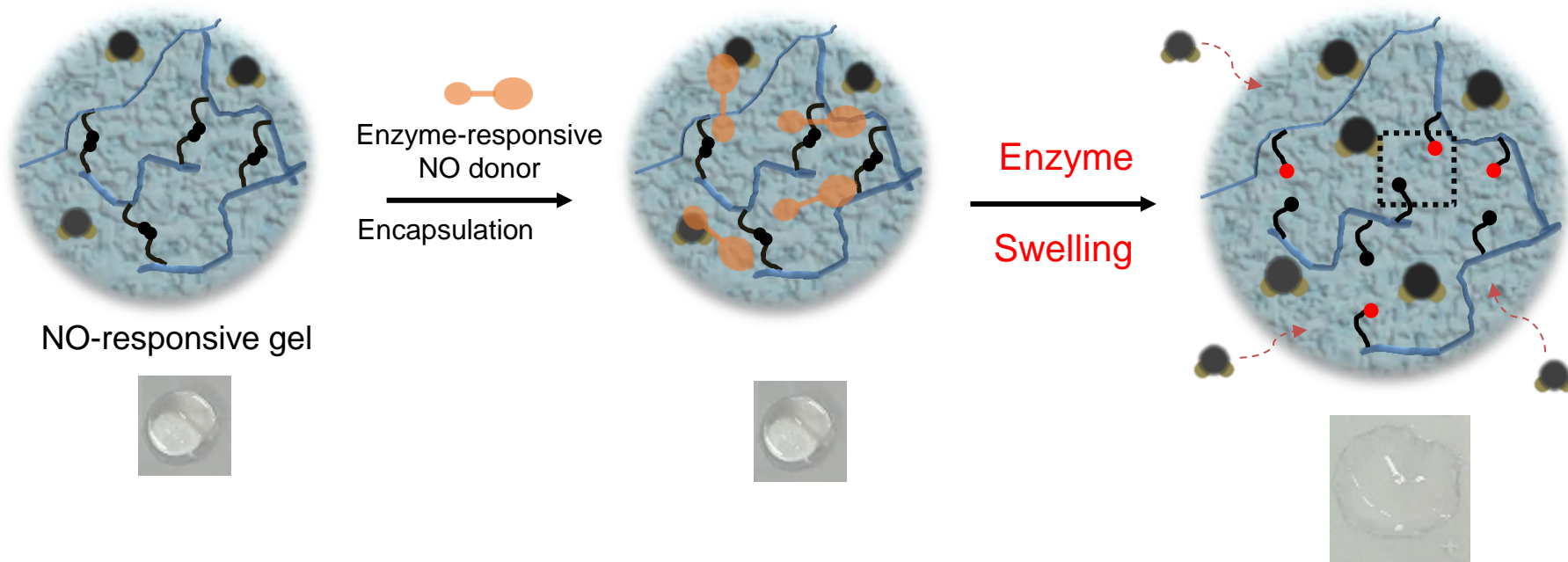
Control gel

NO-r gel



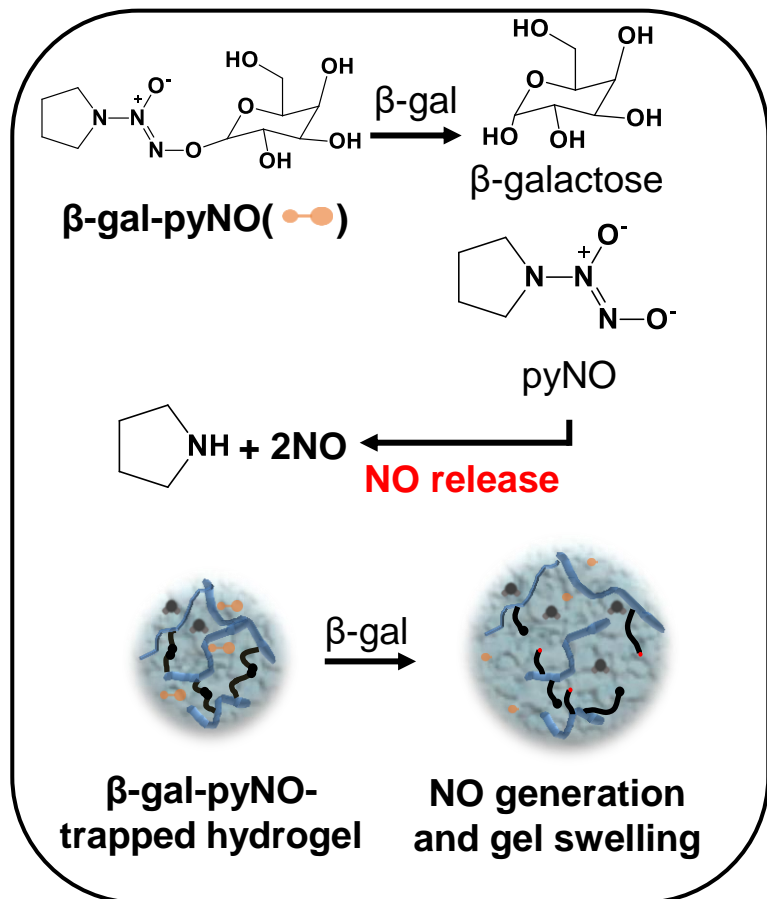
J. Park *et al.* *Advanced Materials*, in press.

Enzyme-responsive gel transformation

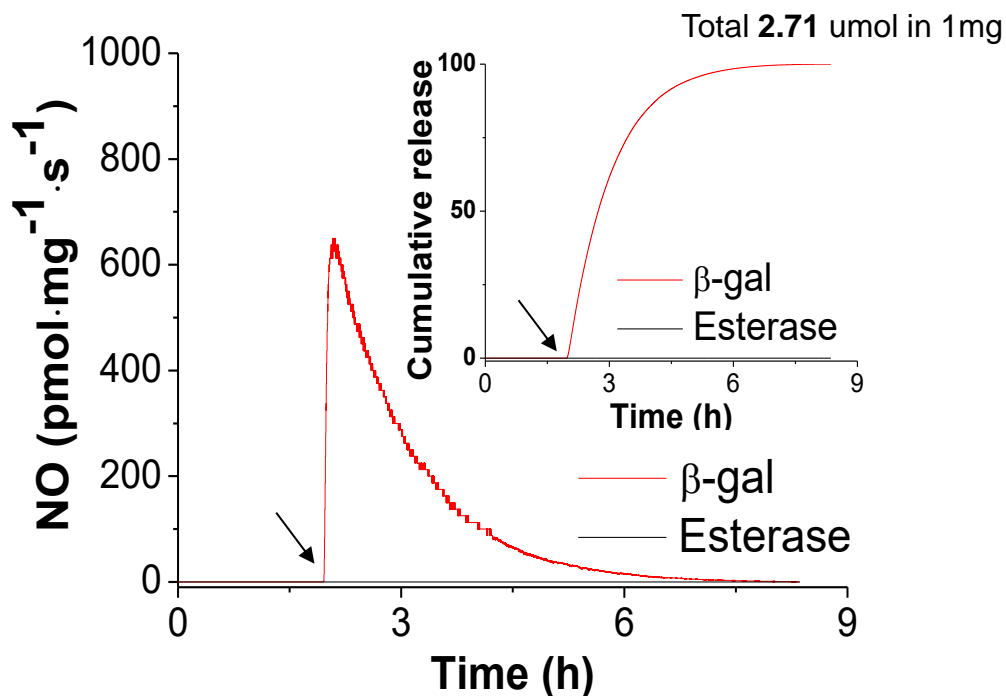


Swelling Test using NO Donor

NO donor : β -gal-pyNO



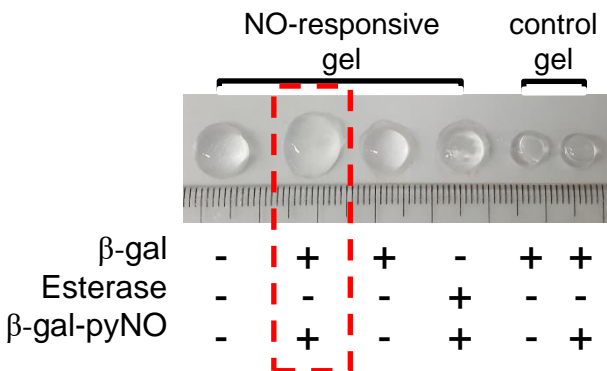
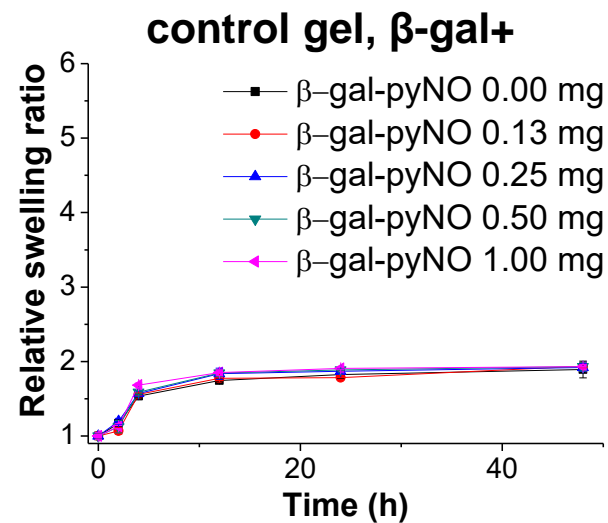
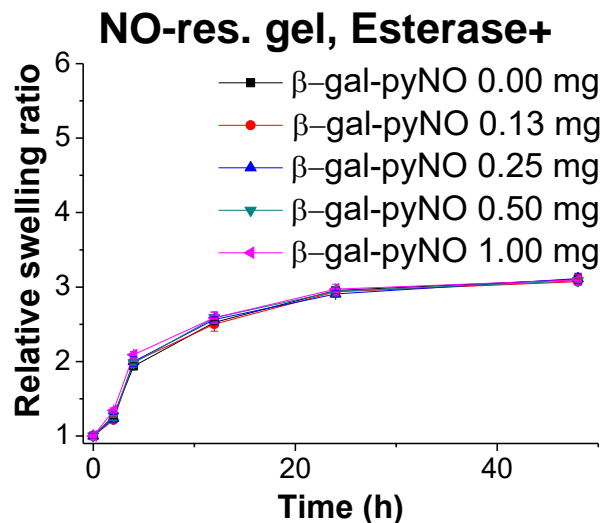
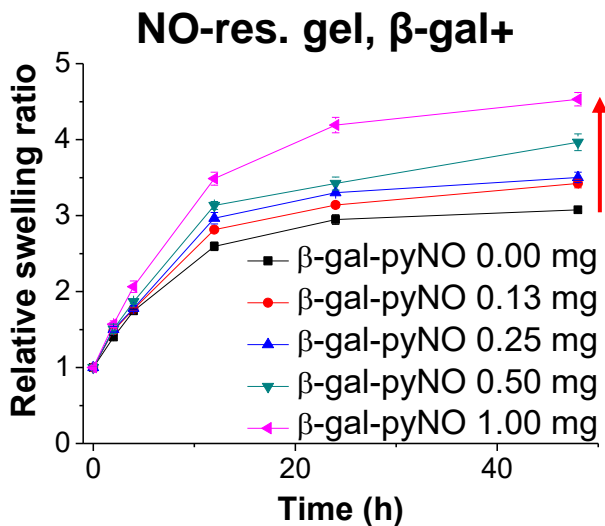
NO releasing profile



β -gal-pyNO can supply a sufficient amount of NO for cleavage of crosslinker

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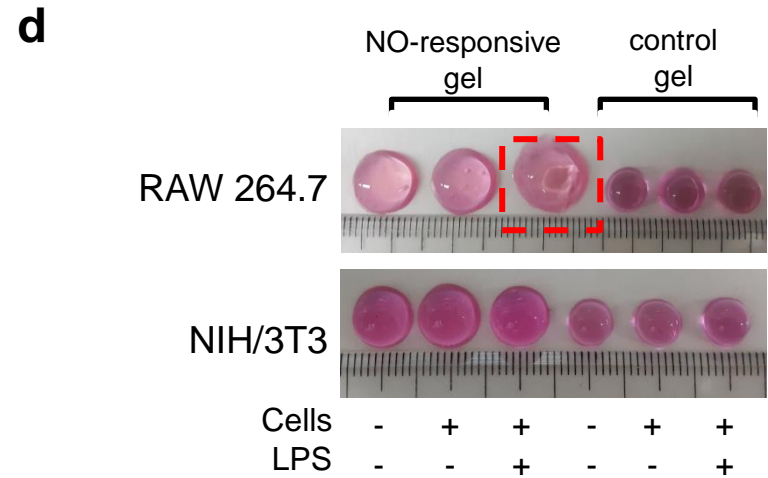
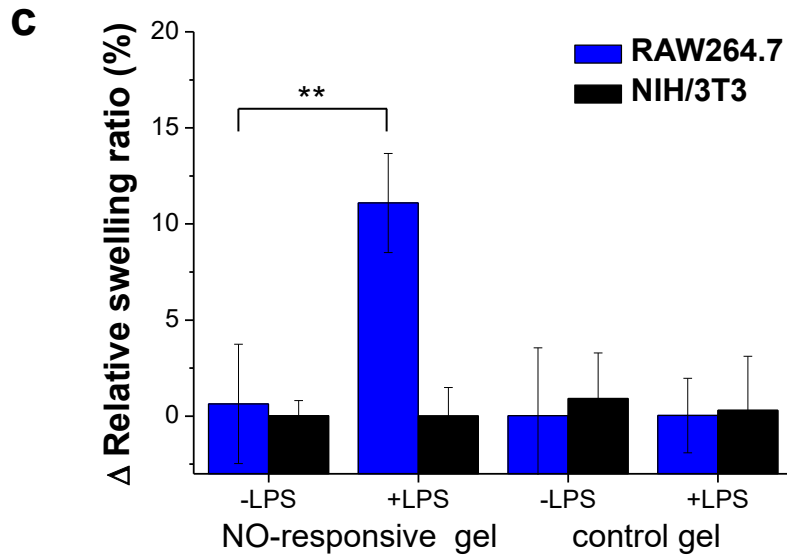
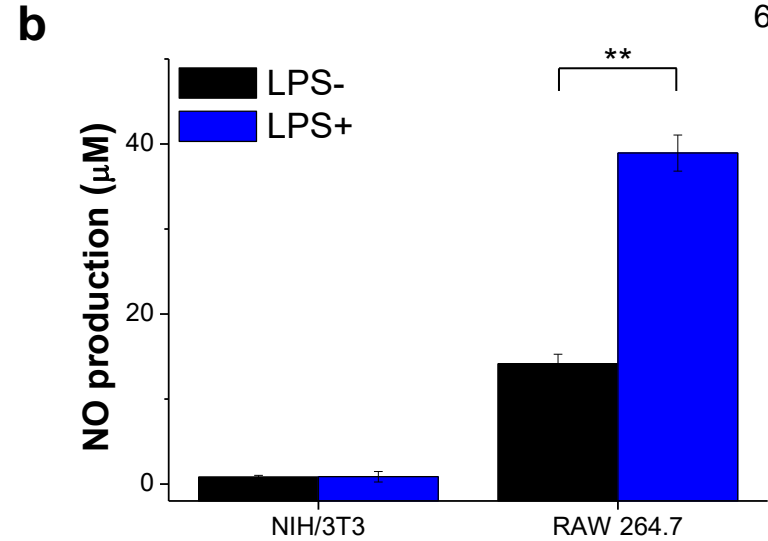
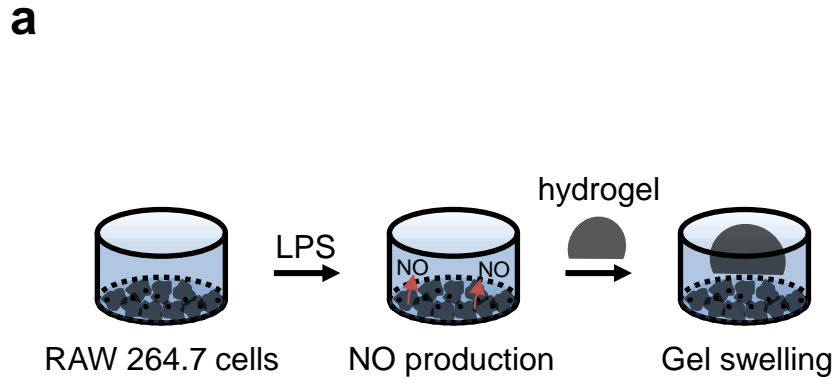
Swelling Test using NO Donor



Enzyme-responsive hydrogel was successfully obtained by encapsulation of enzyme-triggered NO donor

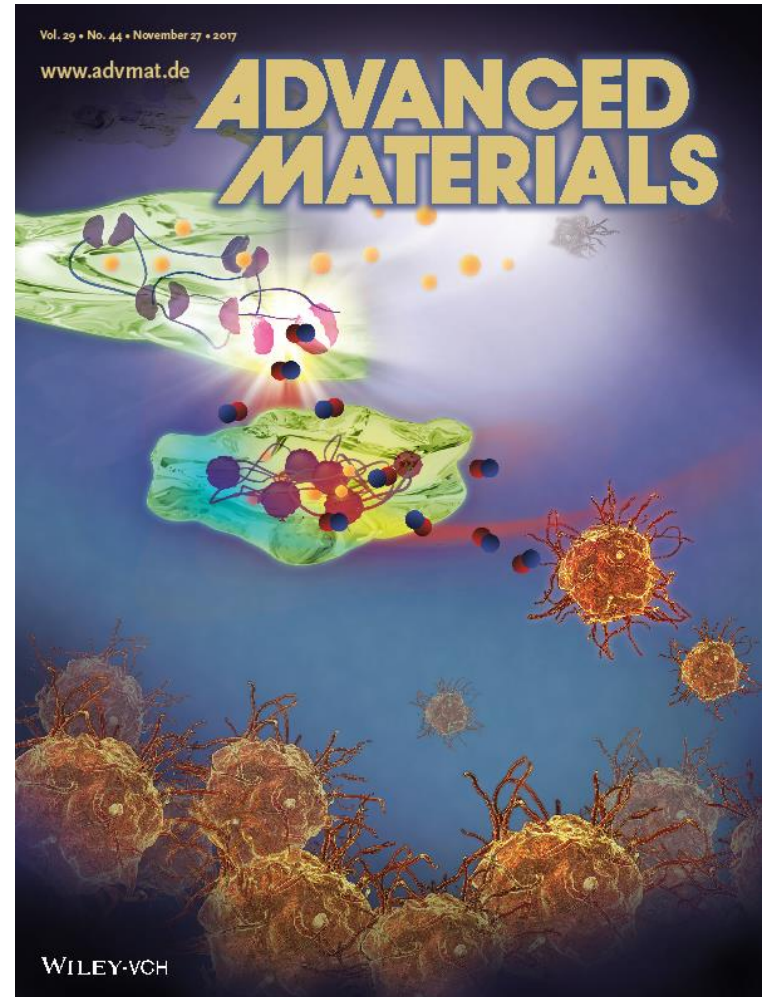
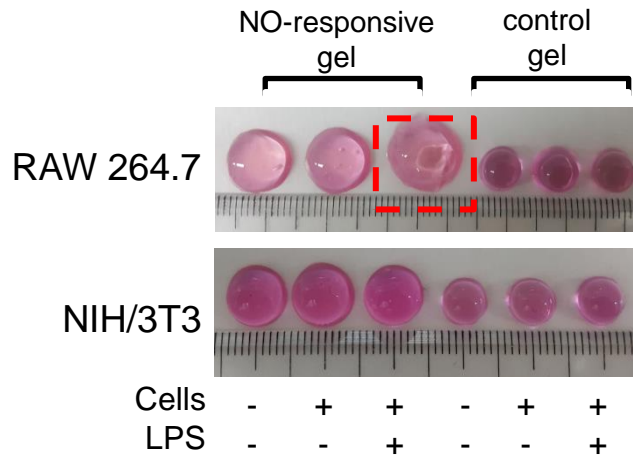
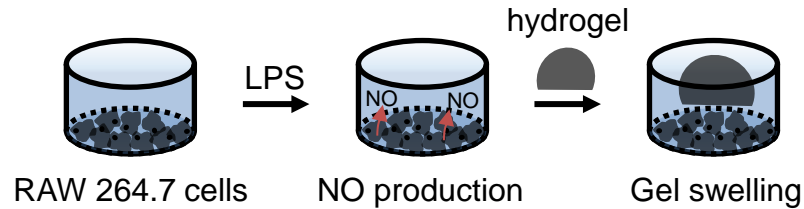
Swelling Test in Macrophage Cells

400,000 cells
6 well plate



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Swelling Test in Macrophage Cells



J. Park *et al.* *Advanced Materials*, 2017, 29, 1702859.

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