

# A PEGylated Bilirubin Nanomedicine for Anticancer and Anti-inflammation Therapy



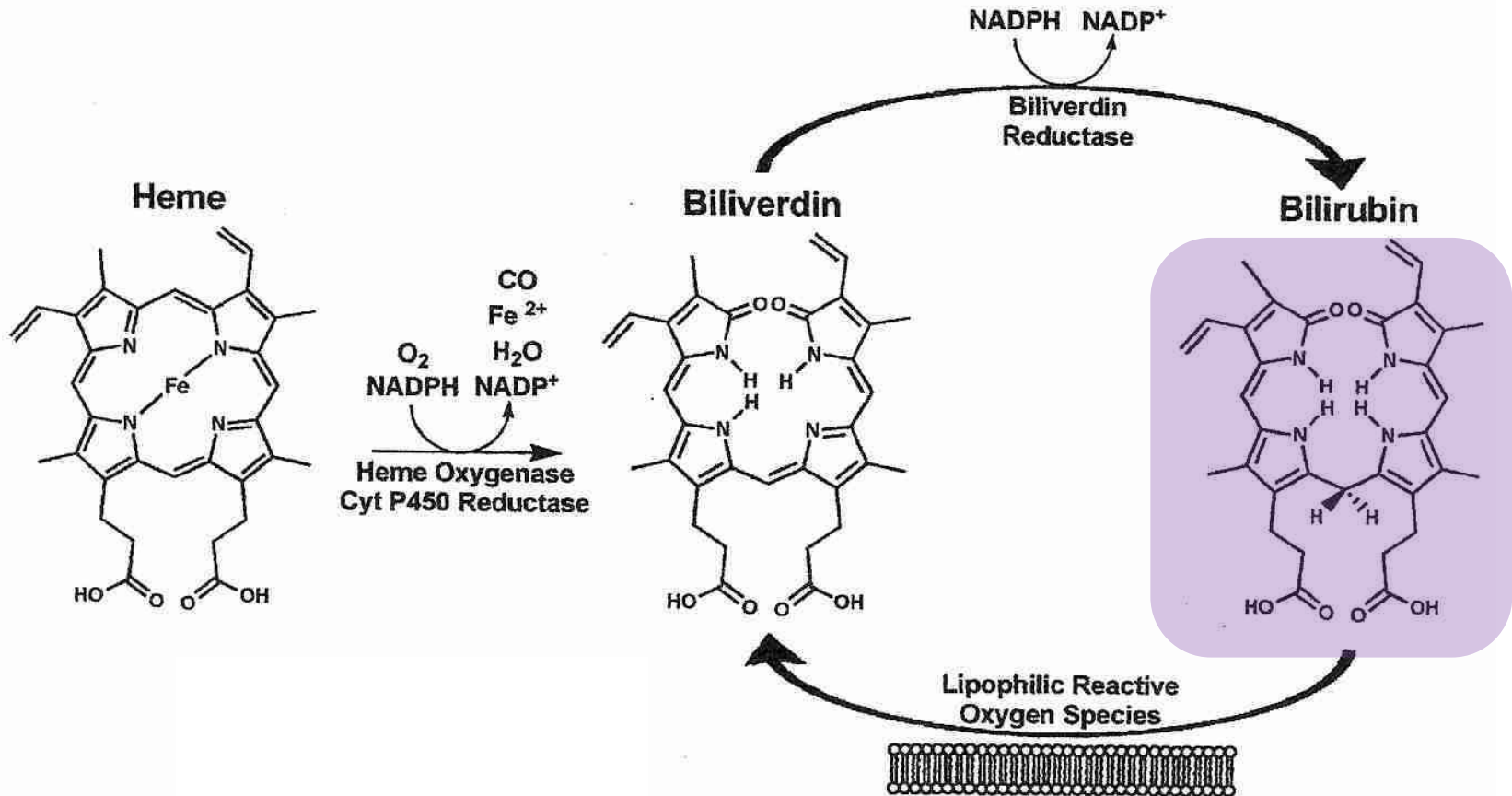
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# Bilirubin? A Final Metabolite of Heme



yellow, bile pigment  
( ~ 1 mg/dL blood)

# Bilirubin as a Bad Guy



**Neonatal jaundice**

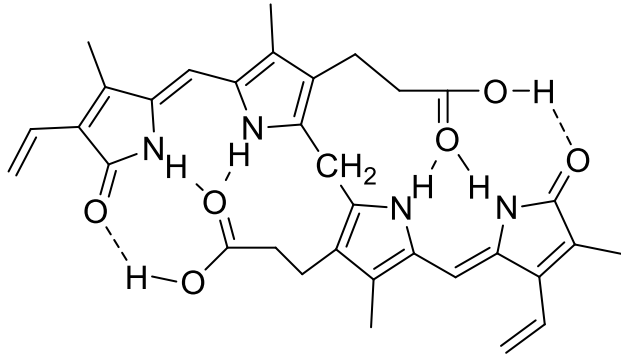


**Eyes with Jaundice  
(liver diseases?)**

**Jaundice itself is not a disease in adults,  
but rather a sign of certain pathological conditions!**



# Bilirubin: Water-insoluble → Jaundice



glucuronyltransferase



in the liver

More water soluble  
'Conjugated' Bilirubin

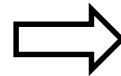
'Unconjugated' Bilirubin (BR)

**Hydrophobic, Water insoluble!**

(yellow colored pigment)



Deposition in various tissues  
(skin, whites of the eyes (sclera), etc)



**Display 'Jaundice' signature**



Eyes with Jaundice

# Epidemiological Study (역학조사) on Bilirubin

- In 1929, Philip Hench, a rheumatologist, made a dramatic observation, correlating relief of incurable symptoms of rheumatoid arthritis with the onset of jaundice.

## **Gilbert syndrome and Ischemic heart disease: a protective effect of elevated bilirubin**

Atherosclerosis (2002) Vol. 160:449–456.

## **Inverse Relationship between serum bilirubin and atherosclerosis in men**

Exp Biol Med. (2003) Vol. 228(5):568-571.

## **Serum bilirubin and inverse correlation with colorectal cancer**

Hepatology (2004) Vol.40: 827- 835.

## **Bilirubin as a Protective Factor for Rheumatoid arthritis**

J Clin Med Res (2010) Vol.2(6):256-260.

## **Relatively higher levels of bilirubin were associated with a lower risk of respiratory disease and all-cause mortality**

JAMA. (2011) Vol. 305(7):691-697.

# Bilirubin: A Potent Anti-oxidant *in Vivo*

Science 27 February 1987:

Vol. 235 no. 4792 pp. 1043-1046

DOI: 10.1126/science.3029864



## **Bilirubin is an antioxidant of possible physiological importance**

R Stocker, Y Yamamoto, AF McDonagh, AN Glazer, BN Ames

NIAS

## **Bilirubin and glutathione have complementary antioxidant and cytoprotective roles**

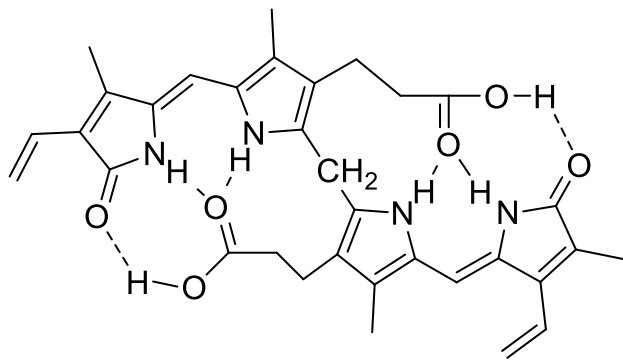
Thomas W. Sedlak<sup>a,b</sup>, Masoumeh Saleh<sup>b</sup>, Daniel S. Higginson<sup>b</sup>, Bindu D. Paul<sup>b</sup>, Krishna R. Juluri<sup>b</sup>, and Solomon H. Snyder<sup>a,b,c,1</sup>

<sup>a</sup>Department of Psychiatry and Behavioral Sciences, <sup>b</sup>The Solomon H. Snyder Department of Neuroscience, and <sup>c</sup>Department of Pharmacology and Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205

*‘Jekyll & ~~Hyde~~’*  
**Character**

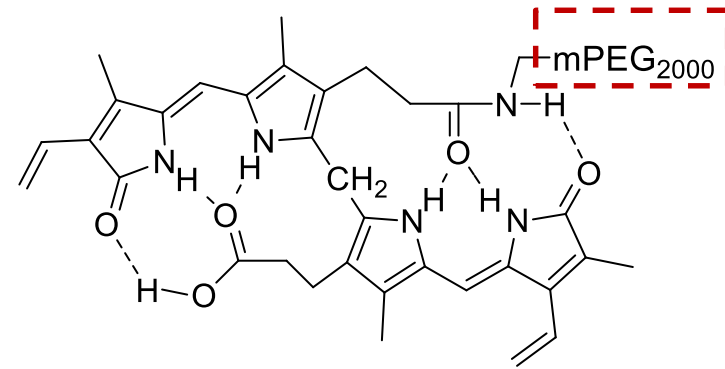
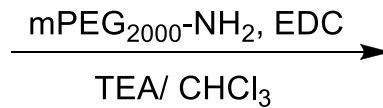
*‘Double-edged Sword’*

# Bilirubin → PEGylated Bilirubin



**Bilirubin**

**Hydrophobic, Water insoluble!**



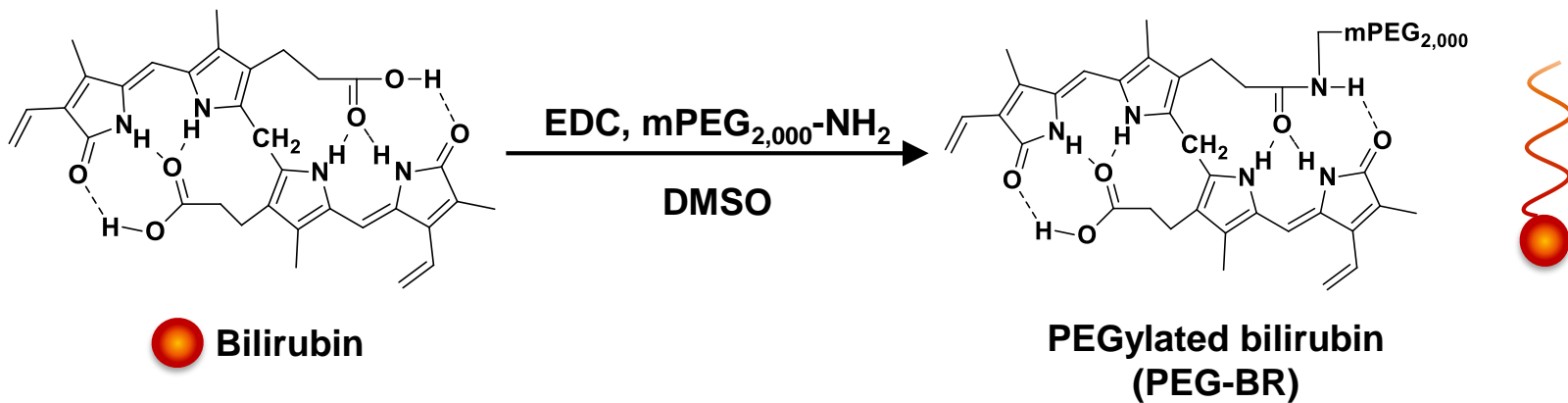
**PEG<sub>2000</sub>-Bilirubin**

**Amphiphilic, Water miscible!**

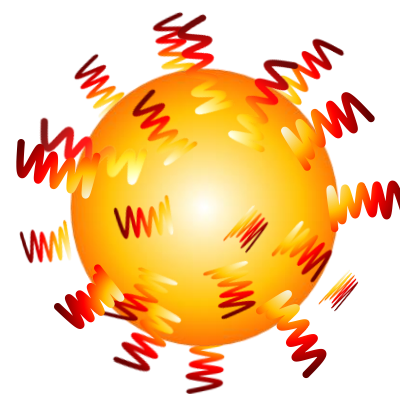
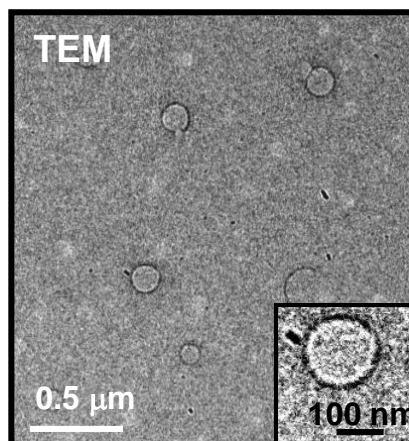
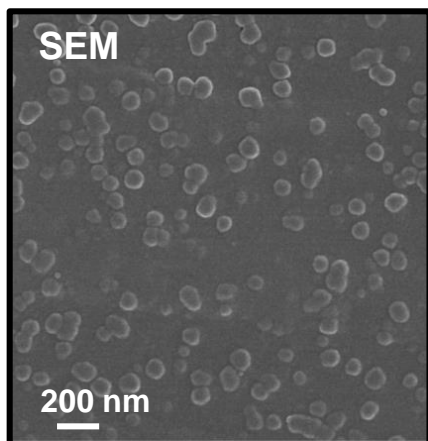
**→ Little chance to be remained or accumulated in the body!**



# Bilirubin Nanoparticles (BRNPs)

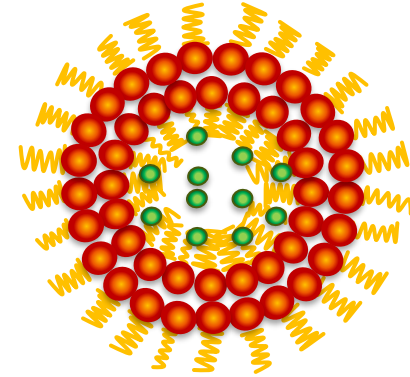
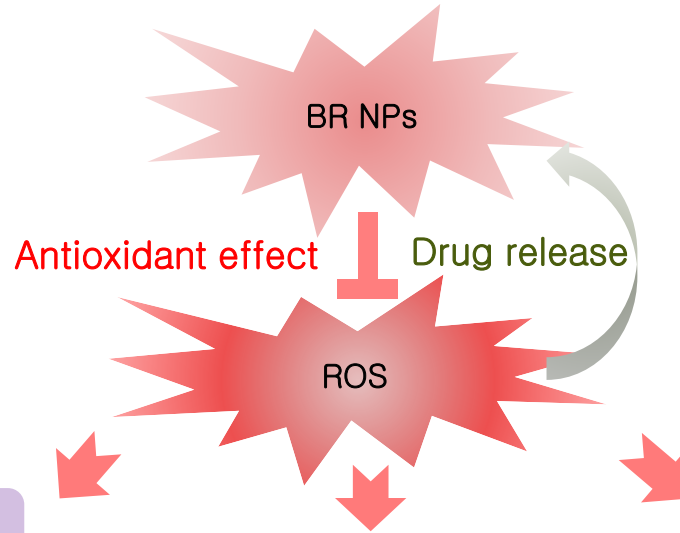
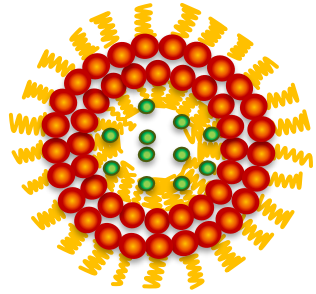


↓ Self-assembly



Bilirubin nanoparticles (BRNPs)

# ROS & Inflammatory Diseases



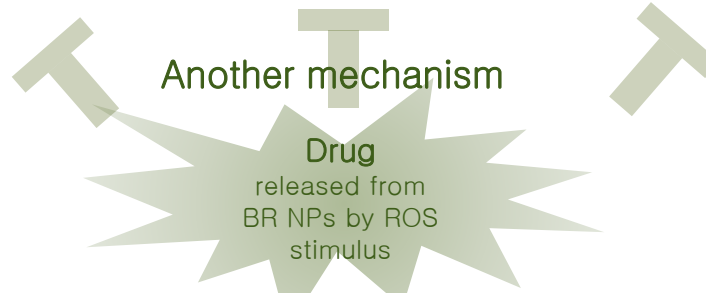
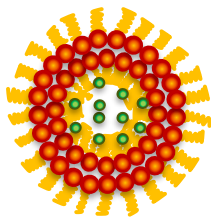
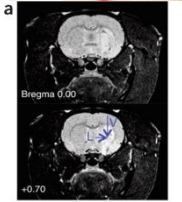
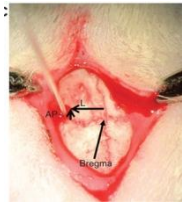
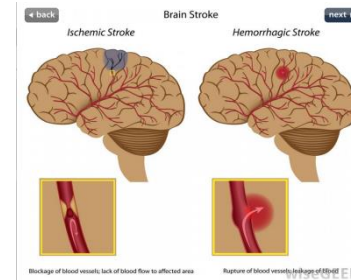
Myocardial infarction model



Acute or Chronic Asthma model



Rat brain ischemic stroke model



# **Bilirubin Nanoparticles: Universal anti-inflammatory therapeutics without immune suppression?**

**1) Inflammatory Bowel Disease: Acute colitis model**

*(Angew Chem Int Ed., 2016)*

**2) Hepatic ischemia-reperfusion injury:**

**Liver transplantation model** *(Biomaterials, 2017)*

**3) Islet xeno-transplantation** *(Biomaterials, 2017)*

**4) Acute asthma** *(Biomaterials, 2017)*

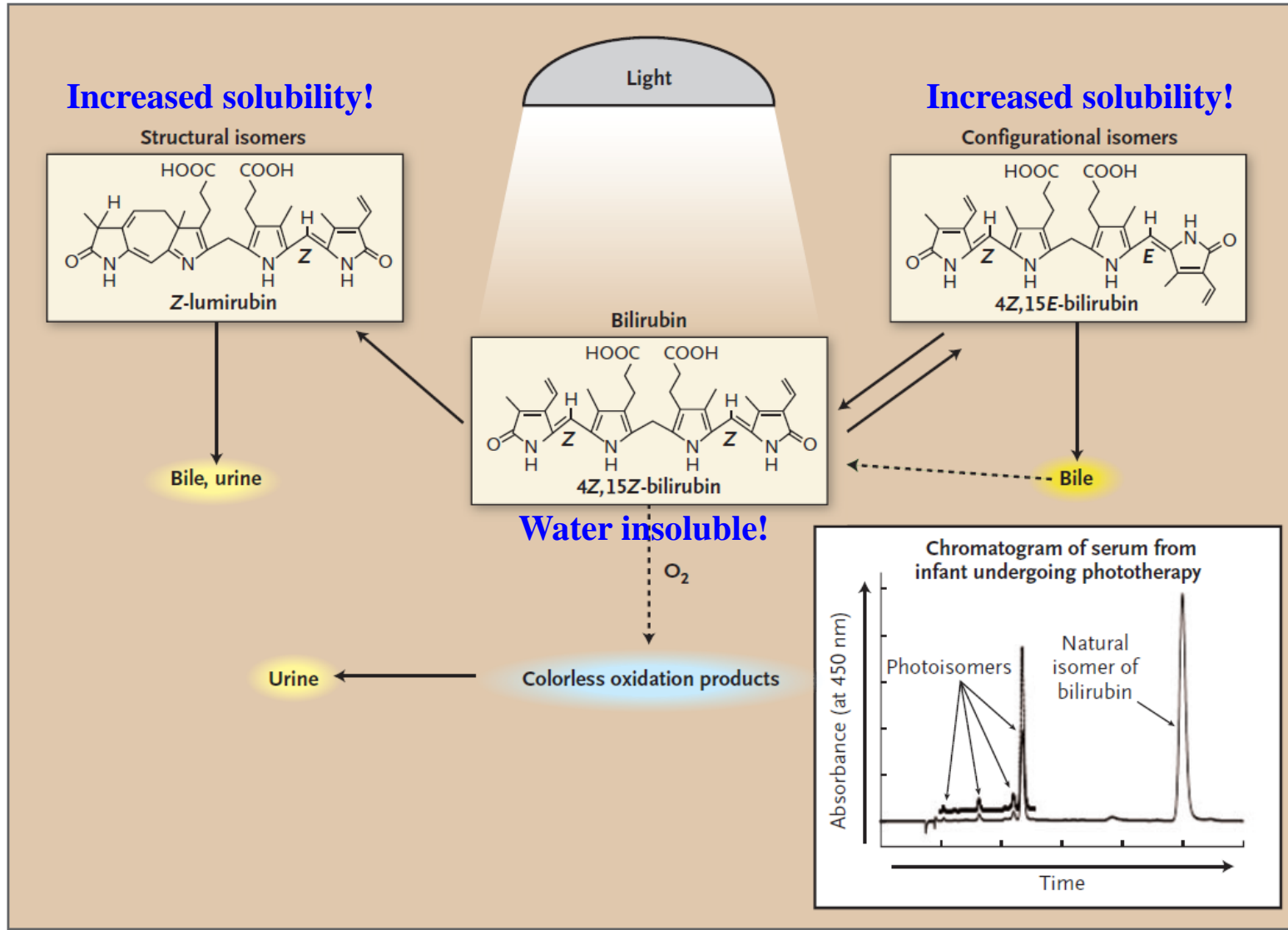
**BRNPs: ROS/Light-responsive Drug  
Delivery Carriers as well as a Medicine!**

# Phototherapy for Neonatal Jaundice



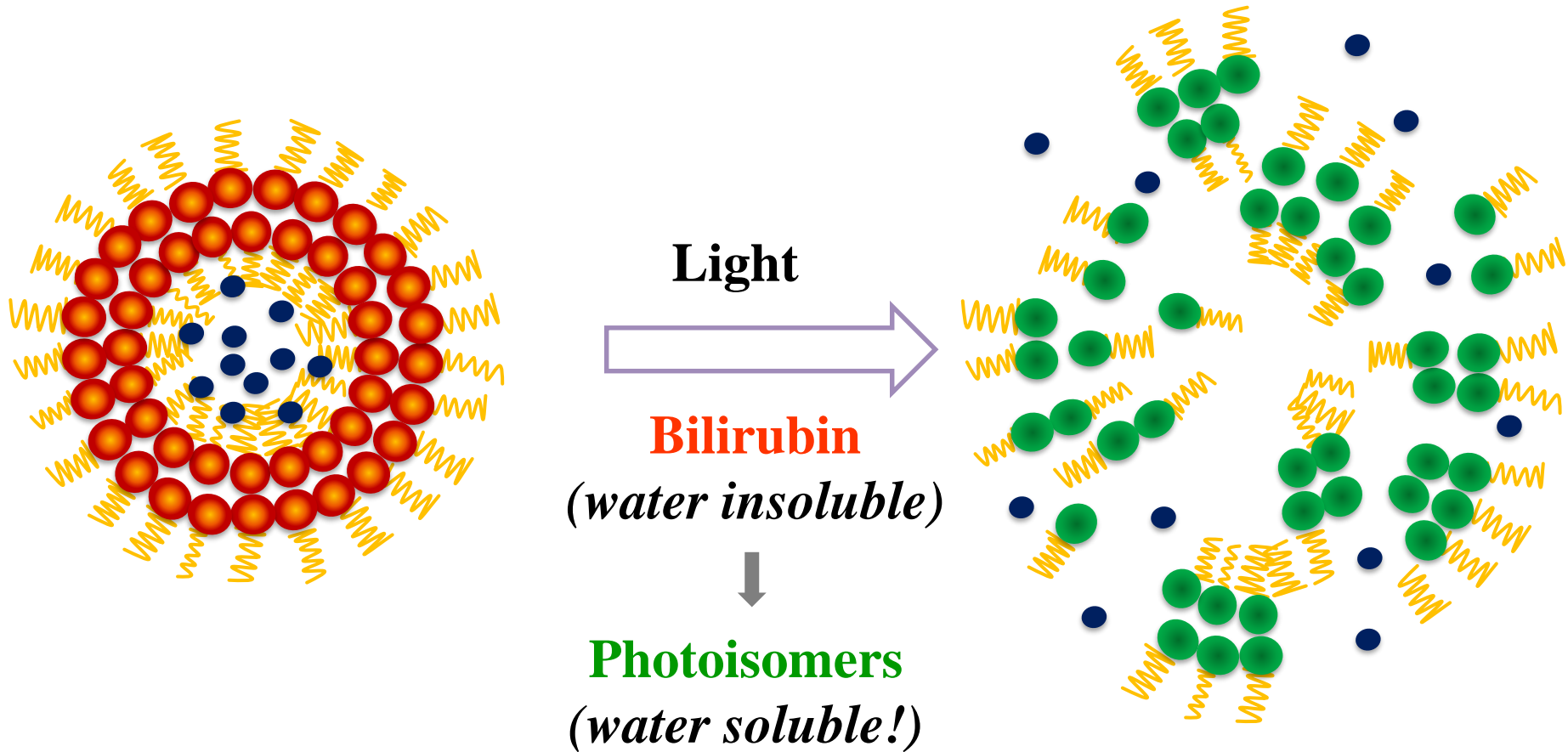
**blue light ~ 450 nm**

# Bilirubin Metabolism During Phototherapy

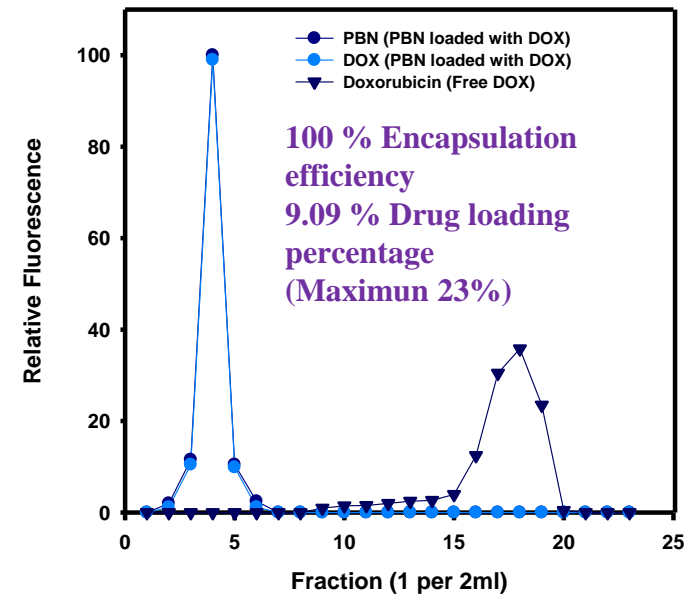
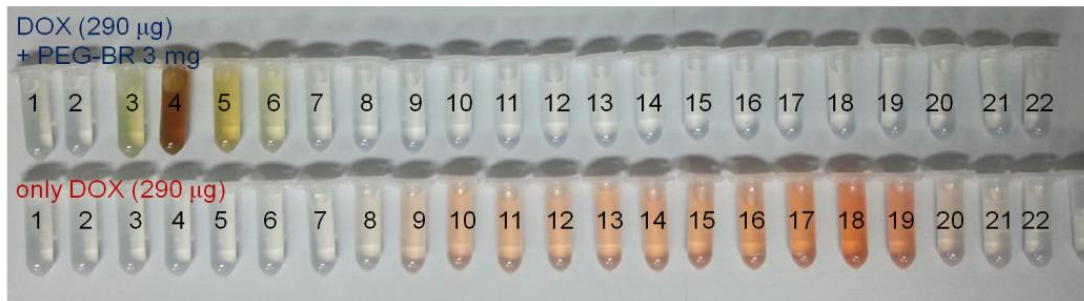
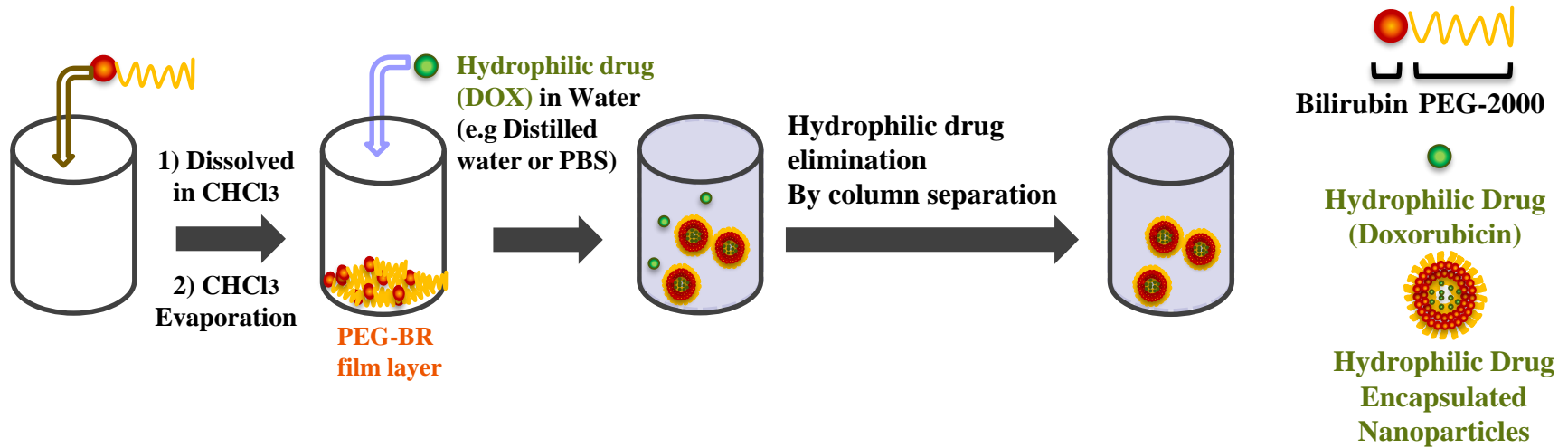




# Light-triggered Disruption of BRNPs



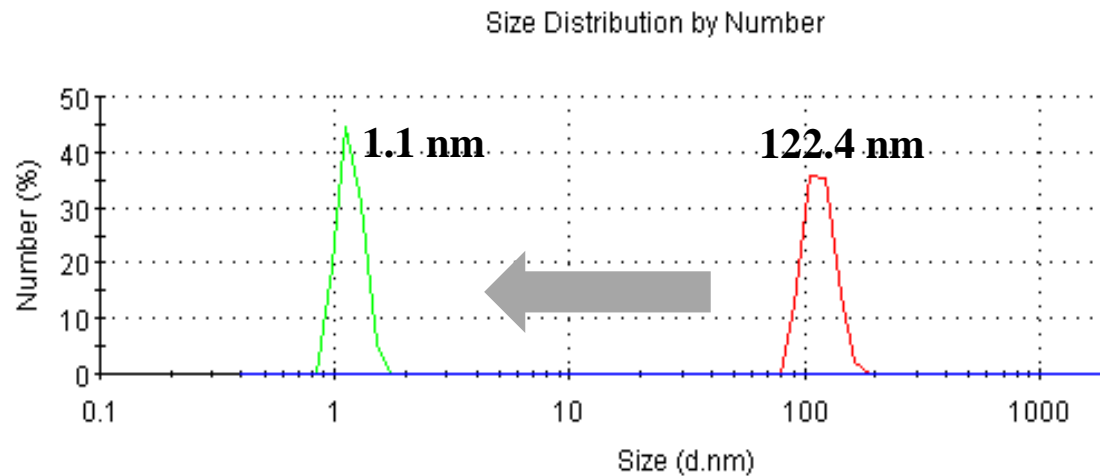
# Drug Loading Capability of BRNPs



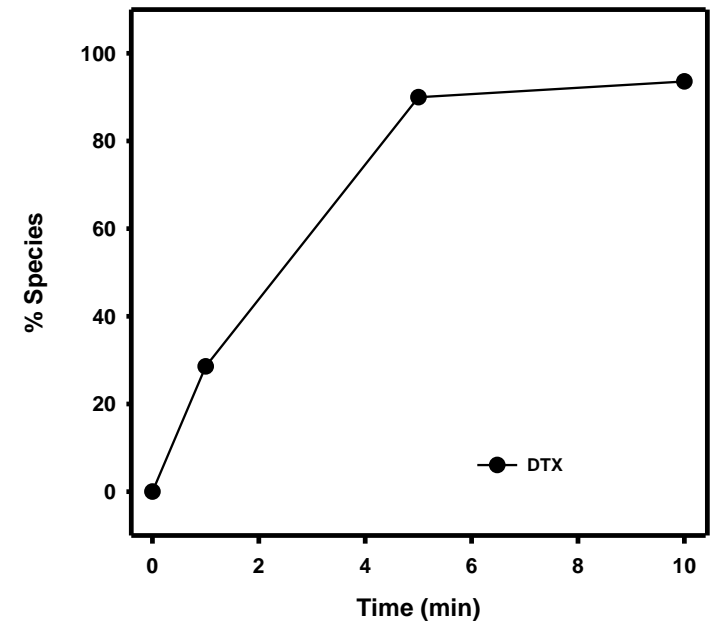
# Light-Induced Disruption of BRNPs

(450nm)

□ Photo irradiation for 1 min at 450 nm  
(10 mW/cm<sup>2</sup>)



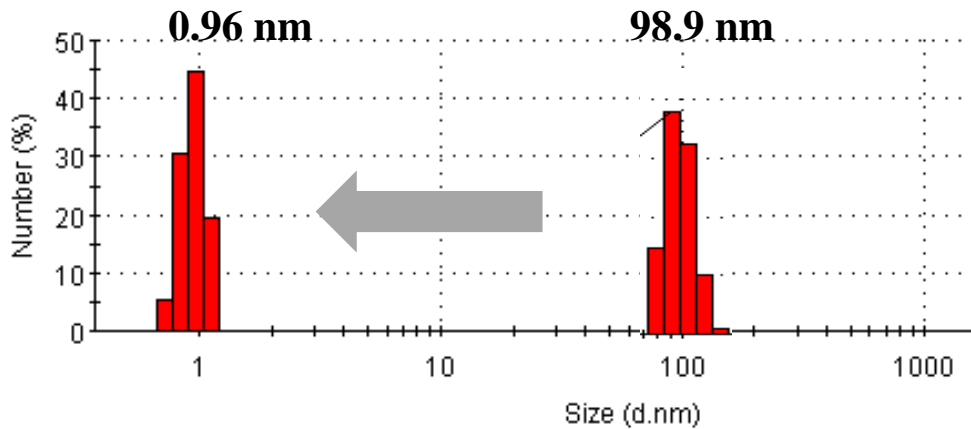
□ Light-induced drug release profile



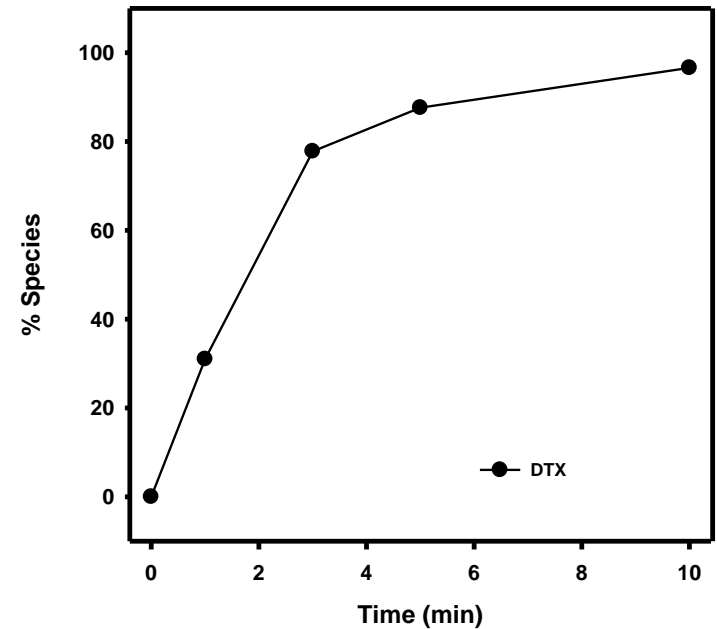
# Light-Induced Disruption of BRNPs

(650nm)

- Photo irradiation for 1 min at 650 nm (90 mW/cm<sup>2</sup>)



- Light-induced drug release profile

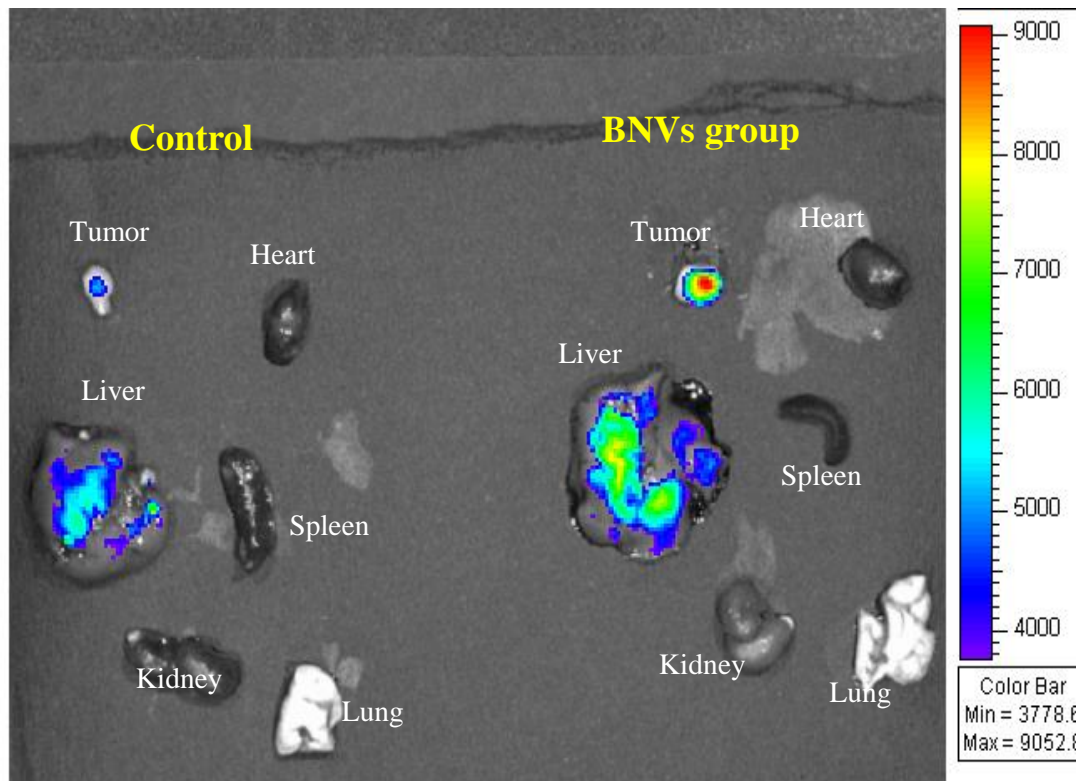


# Cancer Targeting Ability of BRNPs

**Xenograft model : Human lung adenocarcinoma epithelial cell line (A549)  
in Balb/c nude mice**

**Dose: BRNPs (600  $\mu$ g) in PBS, I.V injection**

**Control group: PBS**



# Antitumor Efficacy of BRNPs *in Vivo*

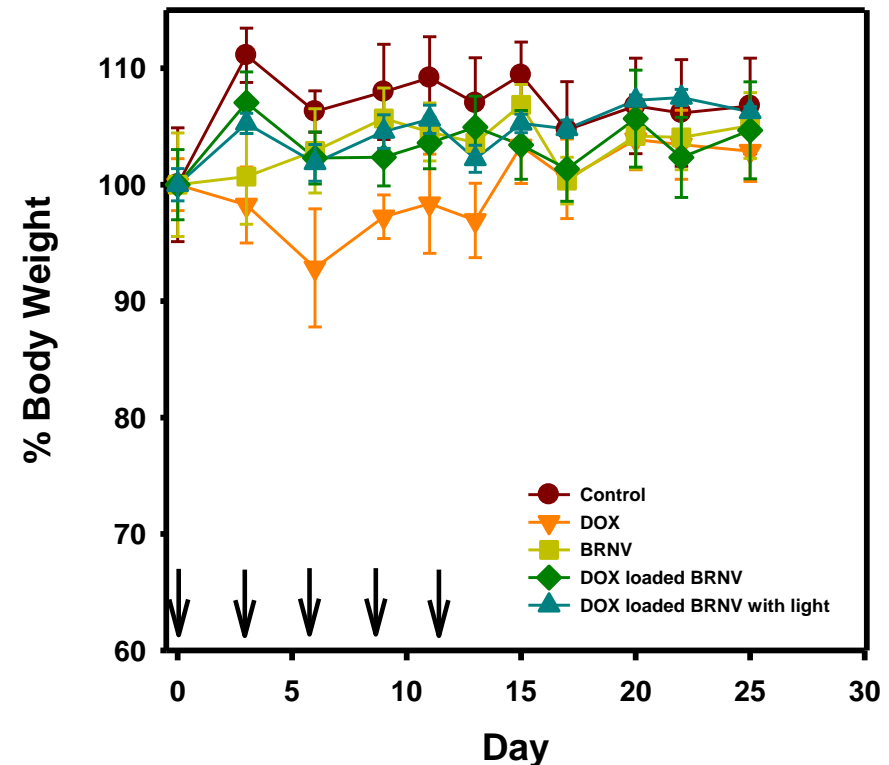
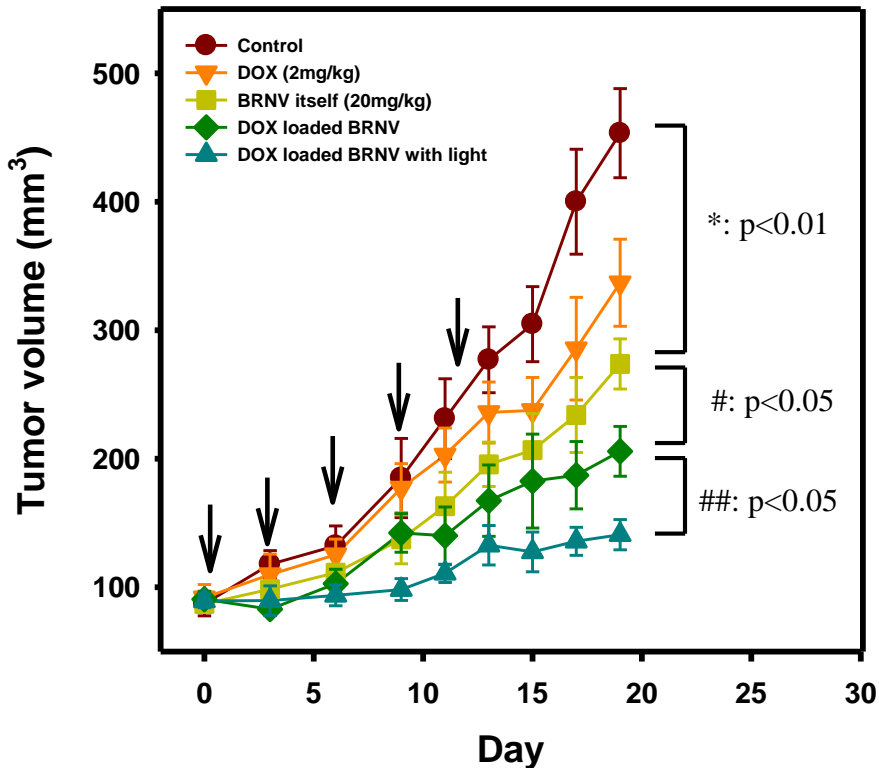


**Mouse** : Balb/c nude mouse 7 weeks

**Tumor** : A549 Human lung carcinoma cell line ( $1 \times 10^6$ )

**Group** : Control group (PBS), DOX (2mg/kg), BRNPs (20mg/kg), DOX(2mg/kg)/BRNPs (20mg/kg), DOX(2mg/kg)/BRNPs (20mg/kg) with laser [Laser : 650nm laser (5min, 200 mW/cm<sup>2</sup>) 30 min after injection of Dox loaded BRNPs]

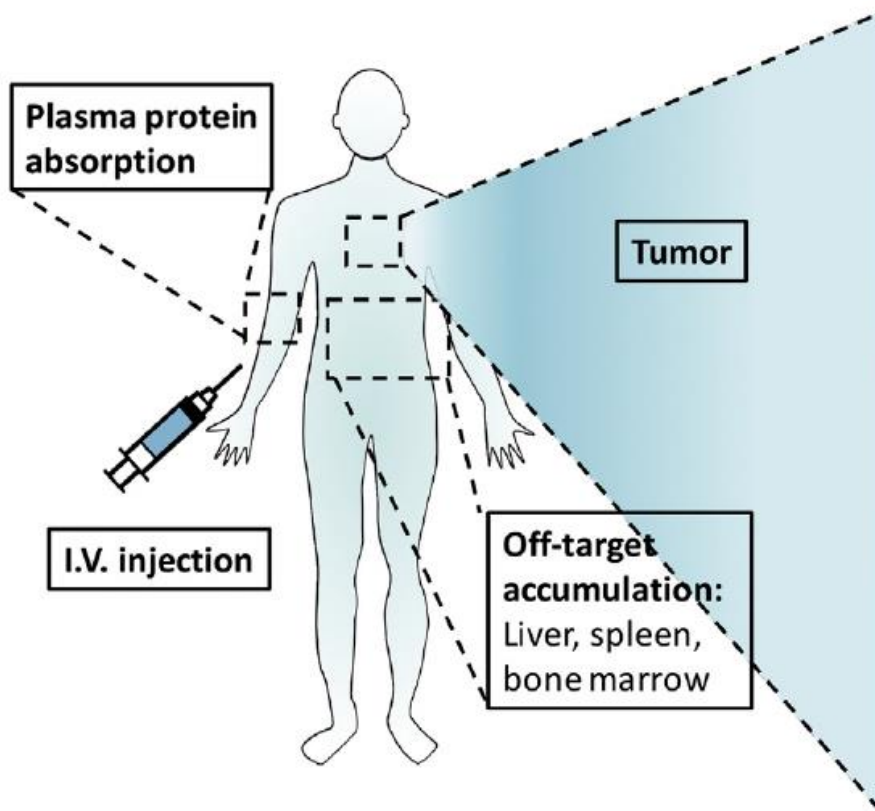
**Dosing schedule** : 0, 3, 6, 9, 12 days



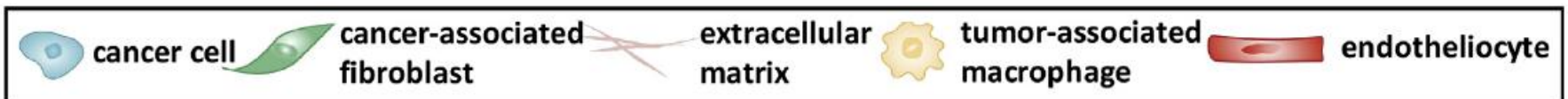
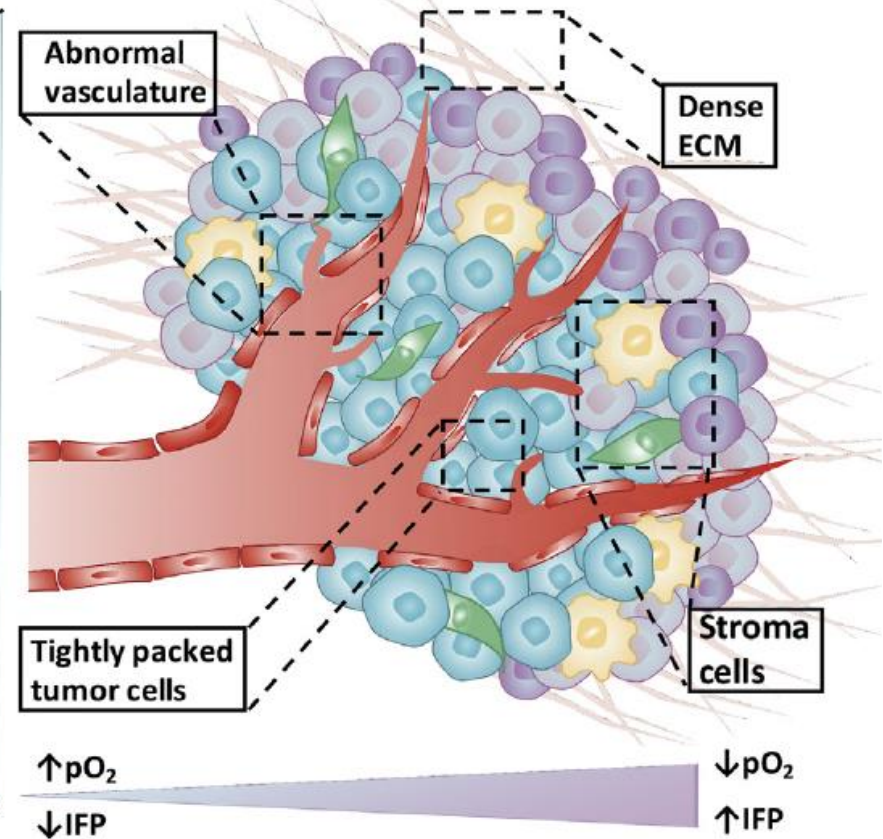


# Tumor microenvironment & nanomedicine

## A. Systemic level



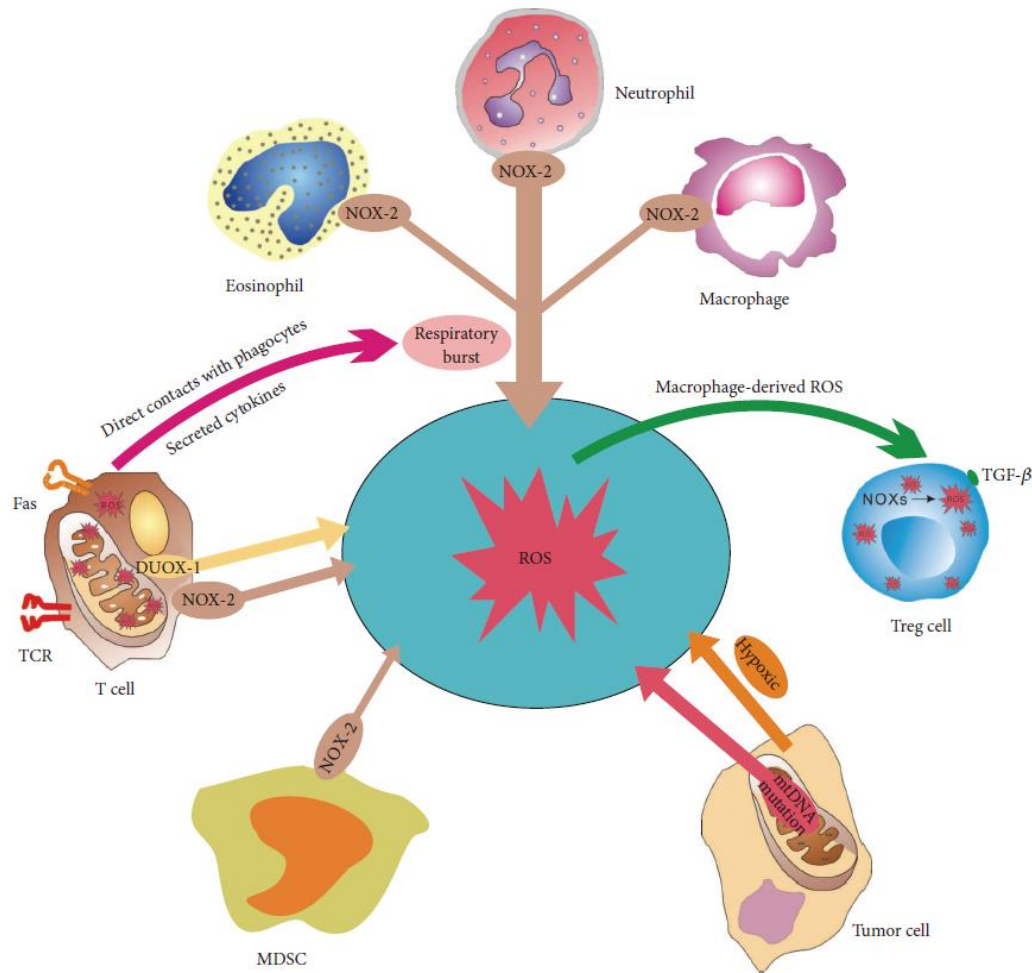
## B. Tumor microenvironment level



Adapted from Overchuk M. and Zheng G., *Biomaterials*, 2018.

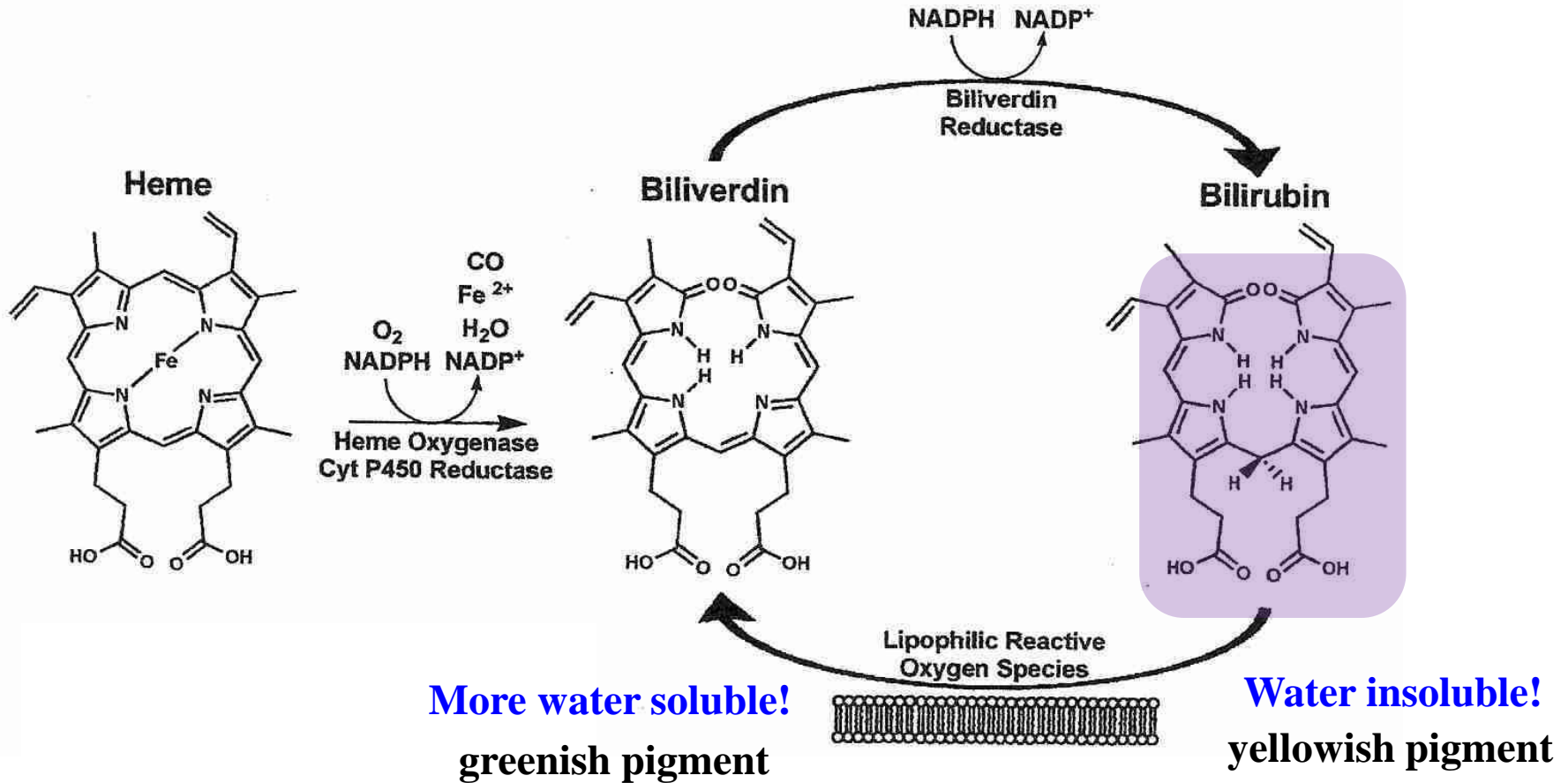
# Tumor microenvironment & ROS

ROS are overproduced in the TME!

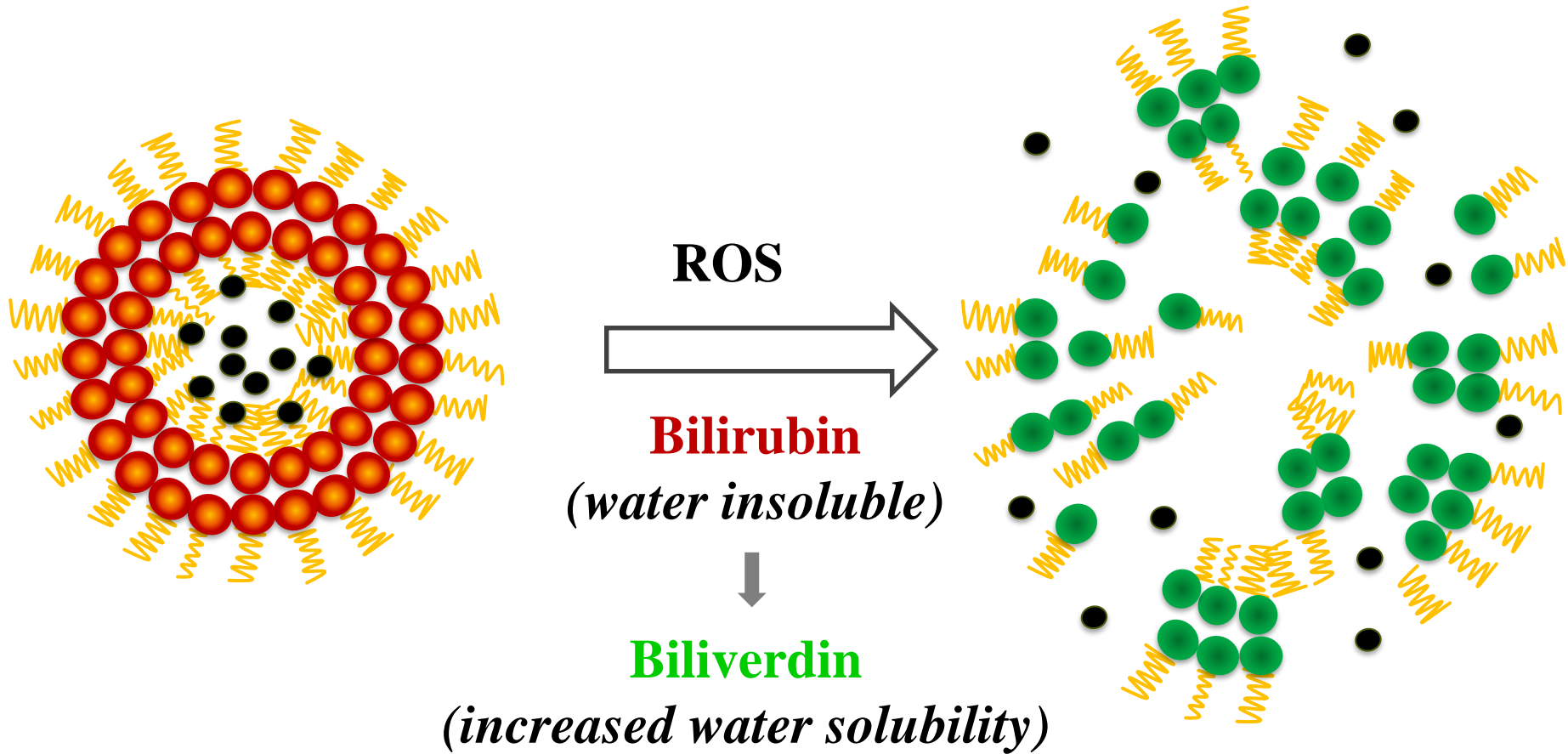


Adapted from Zhang Y. et al., *Oxidative Medicine and Cellular Longevity*, 2016.

# Bilirubin: A Redox Active Compound

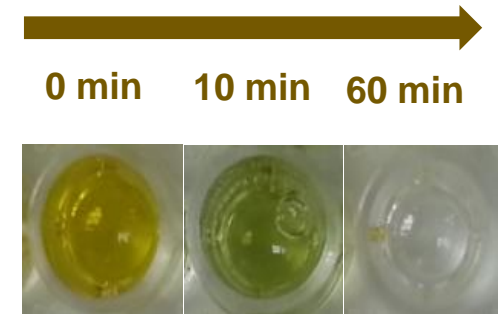
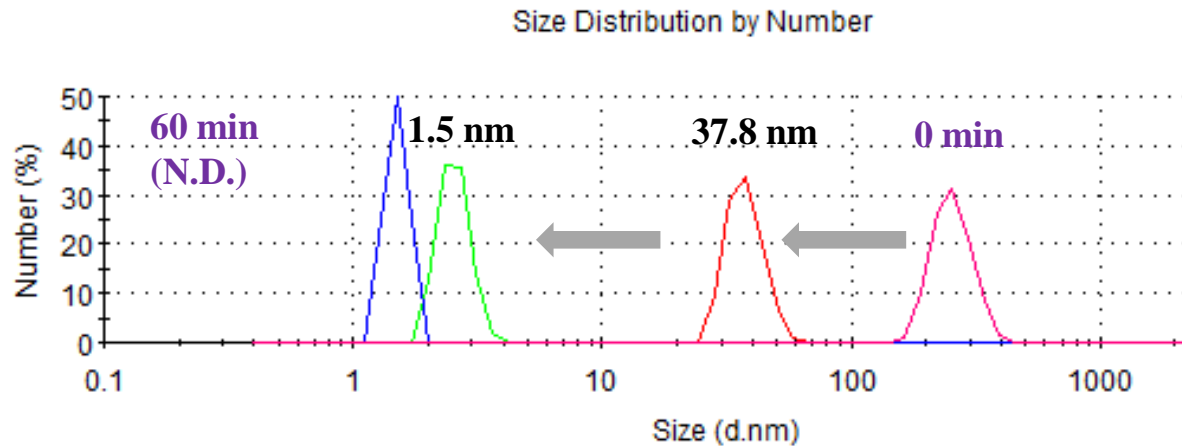


# ROS-triggered Disruption of BRNPs



# ROS-triggered Disruption of BRNPs

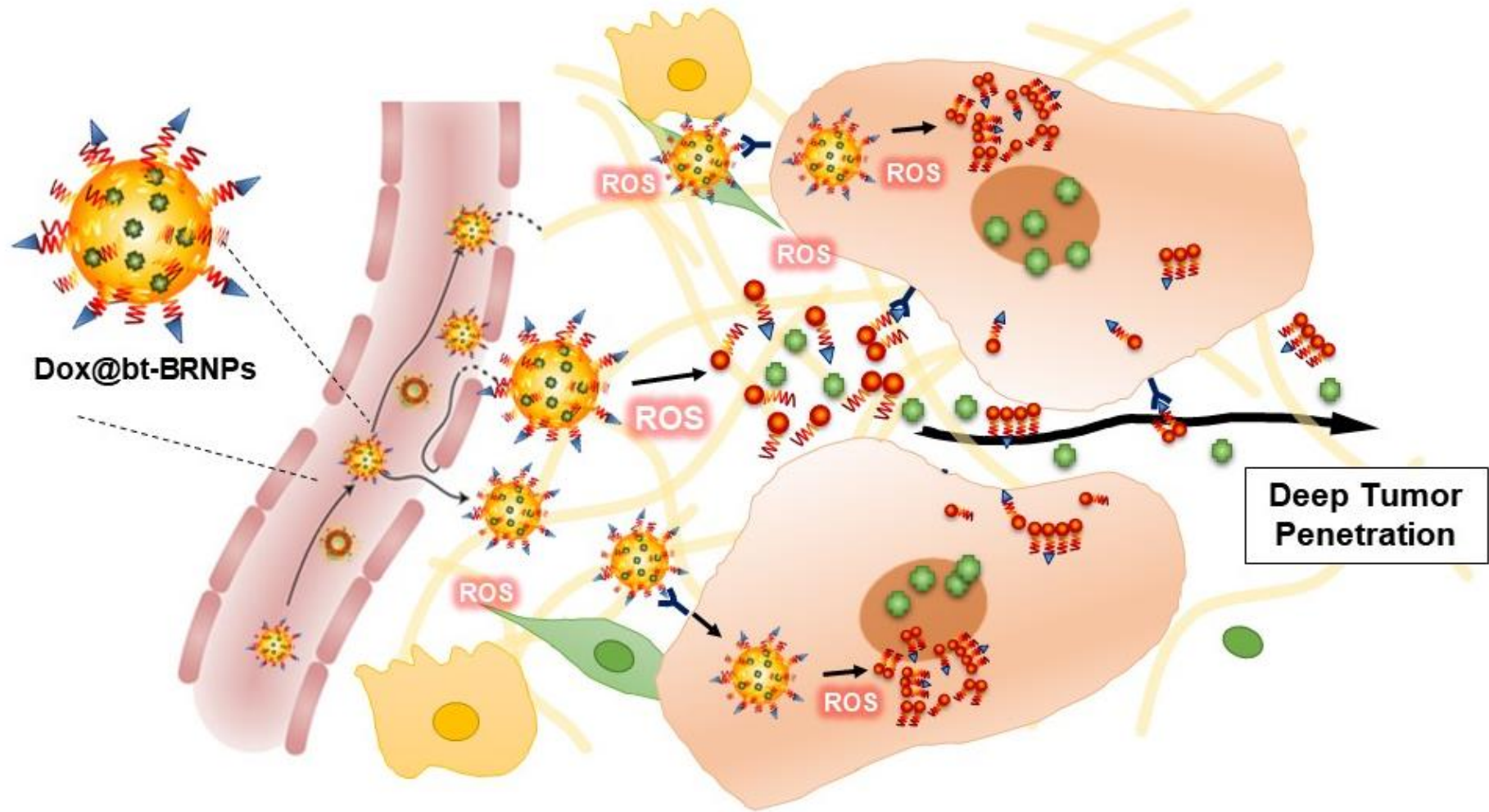
□ Upon oxidation by peroxy radicals



- ➔ The size of BRNPs drastically decreased!
- ➔ Solution color of BRNPs became changed



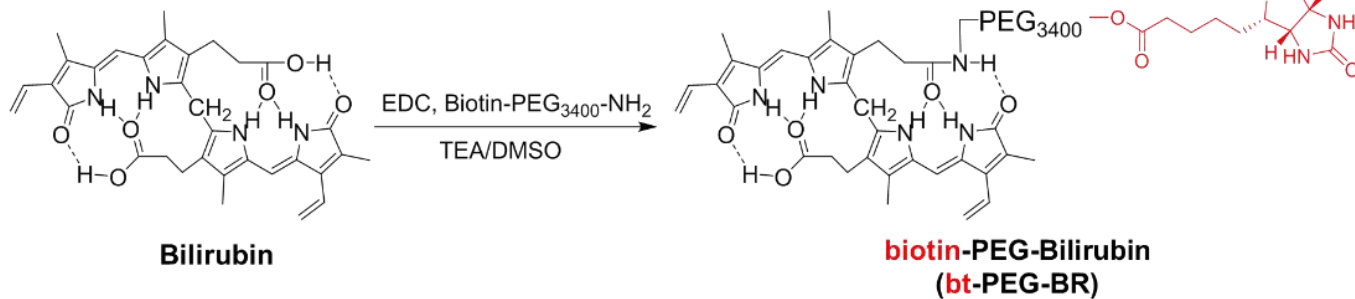
# bt-BRNPs as a TME ROS-targeting nanomedicine



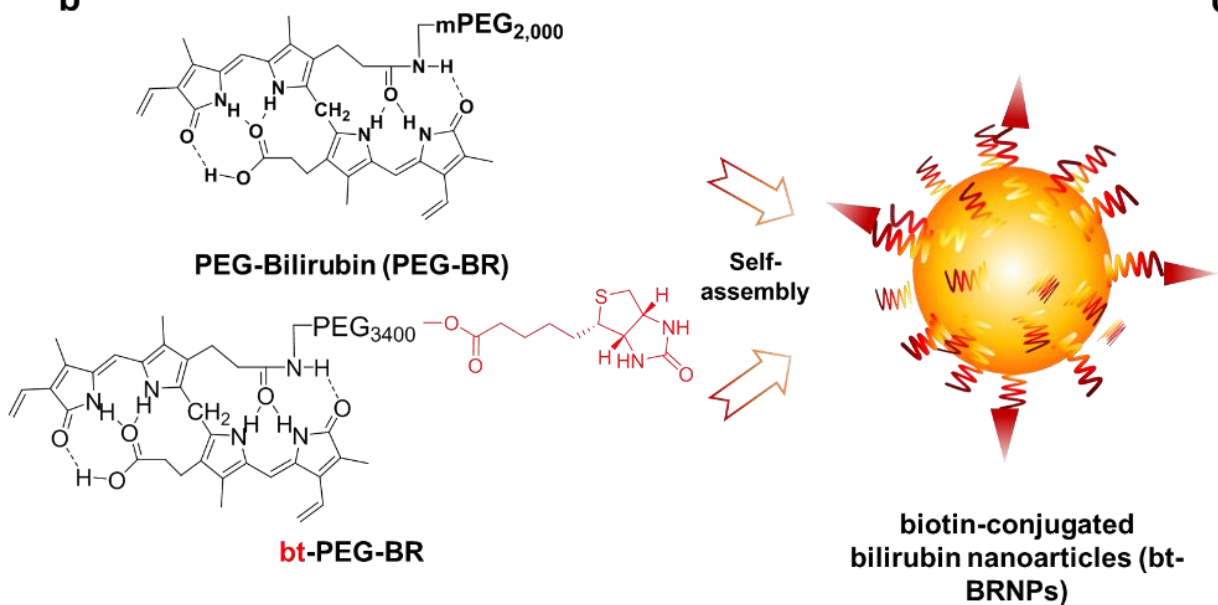


# Preparation of ROS-responsive bt-BRNPs

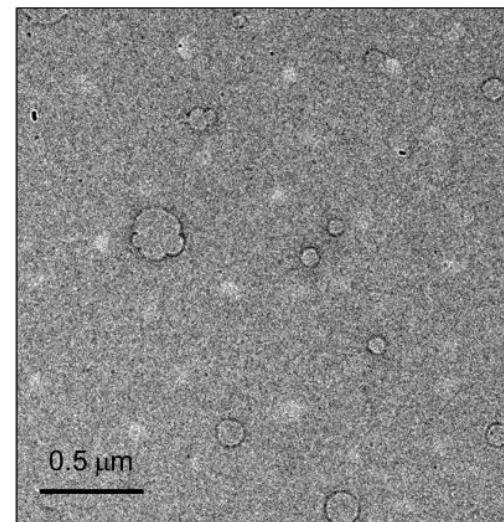
**a**



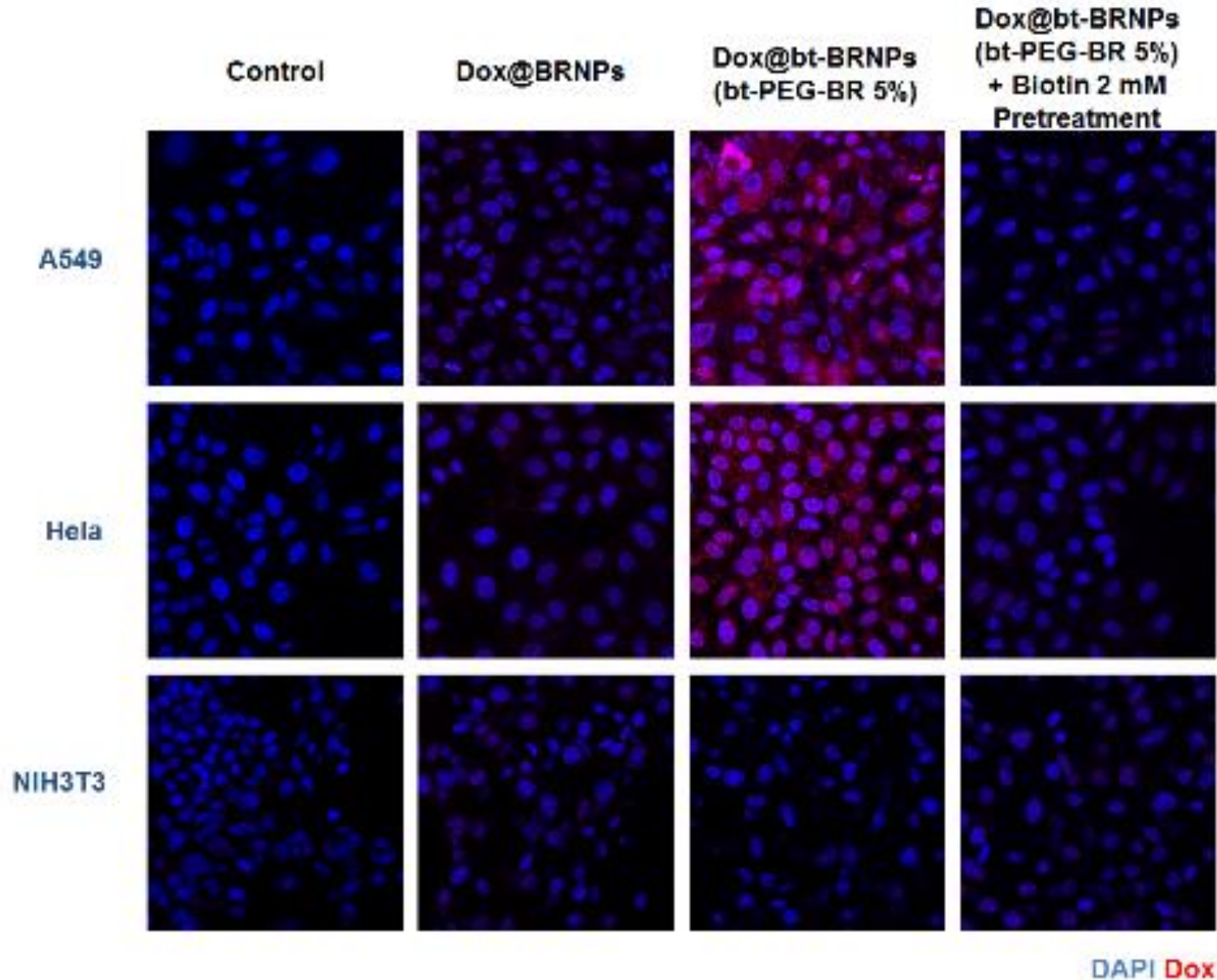
**b**



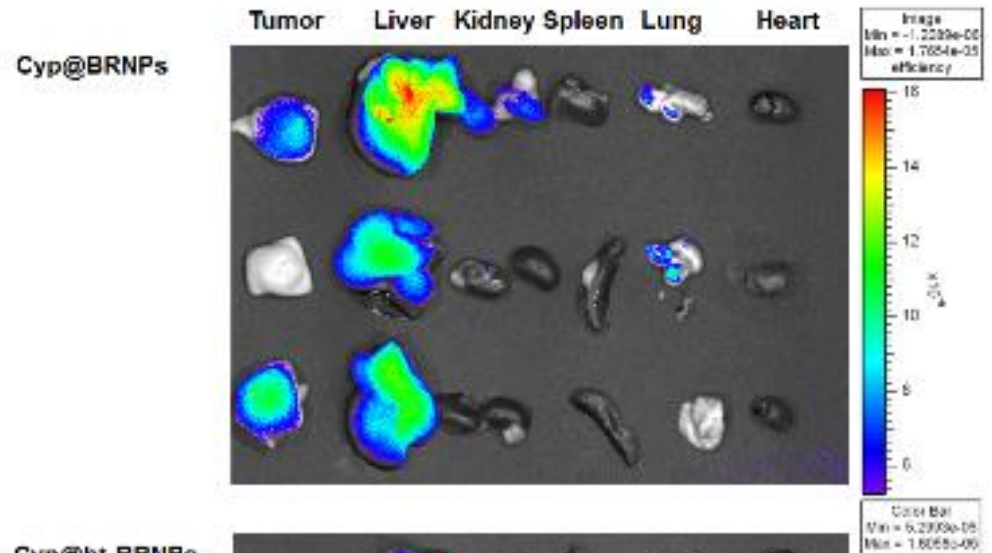
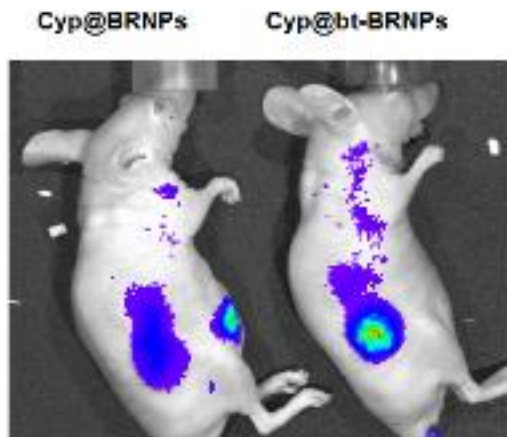
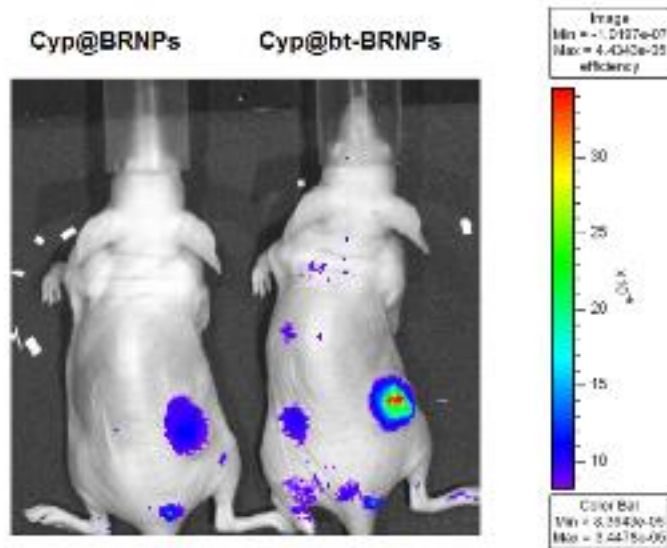
**c**



# Cell targeting and drug release of Dox@bt-BRNPs



# Tumor targeting of Cyp@bt-BRNPs



# Antitumor Efficacy of BRNPs *in Vivo*

