Nanoparticle-Augmented Sonodynamic Therapy

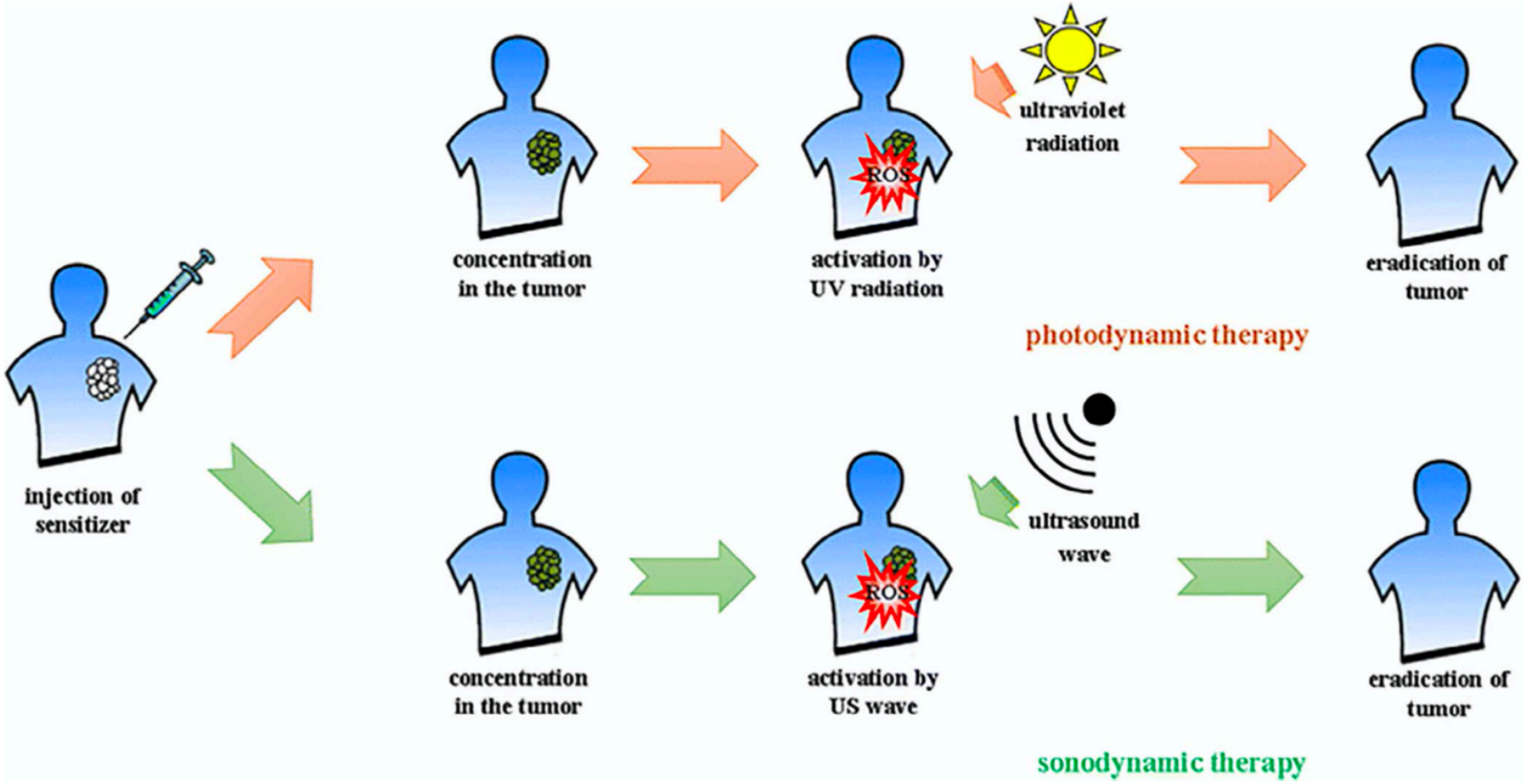
Literature Seminar

2020/6/1

Wataru Atsumi (B4)

Contents

Photodynamic therapy (PDT) & Sonodynamic therapy (SDT)



PDT

- Limited penetration depth

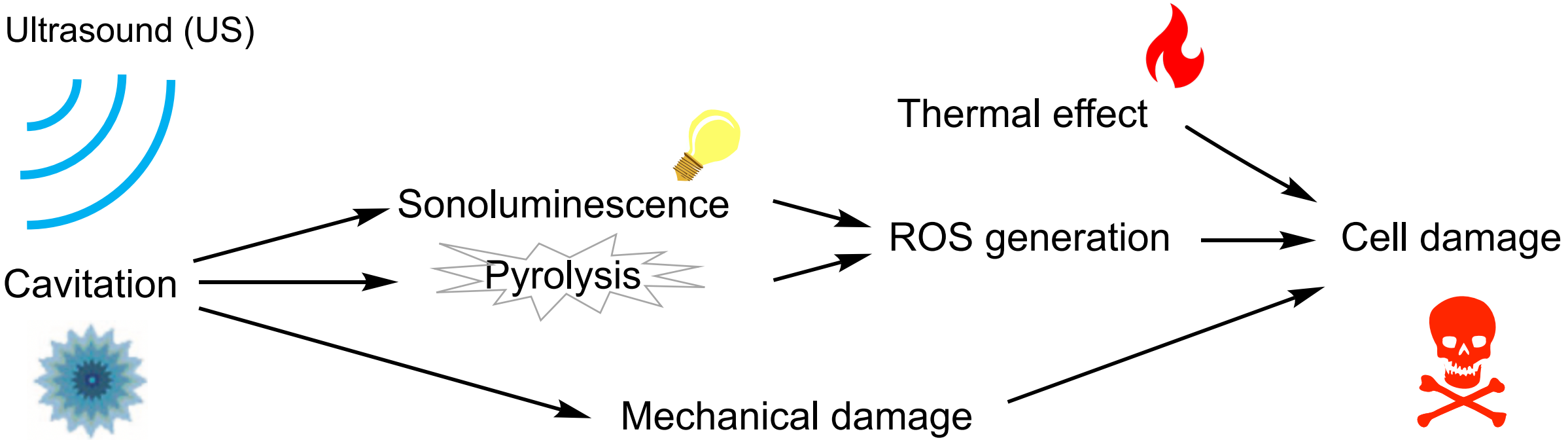
SDT

- High penetration depth
- Less invasive

Chemical Engineering Journal, **2018**, *340*, 155–172

Possible mechanisms of SDT

Schematic diagram of the possible mechanism of SDT



Possible mechanisms of SDT

1. Cavitation

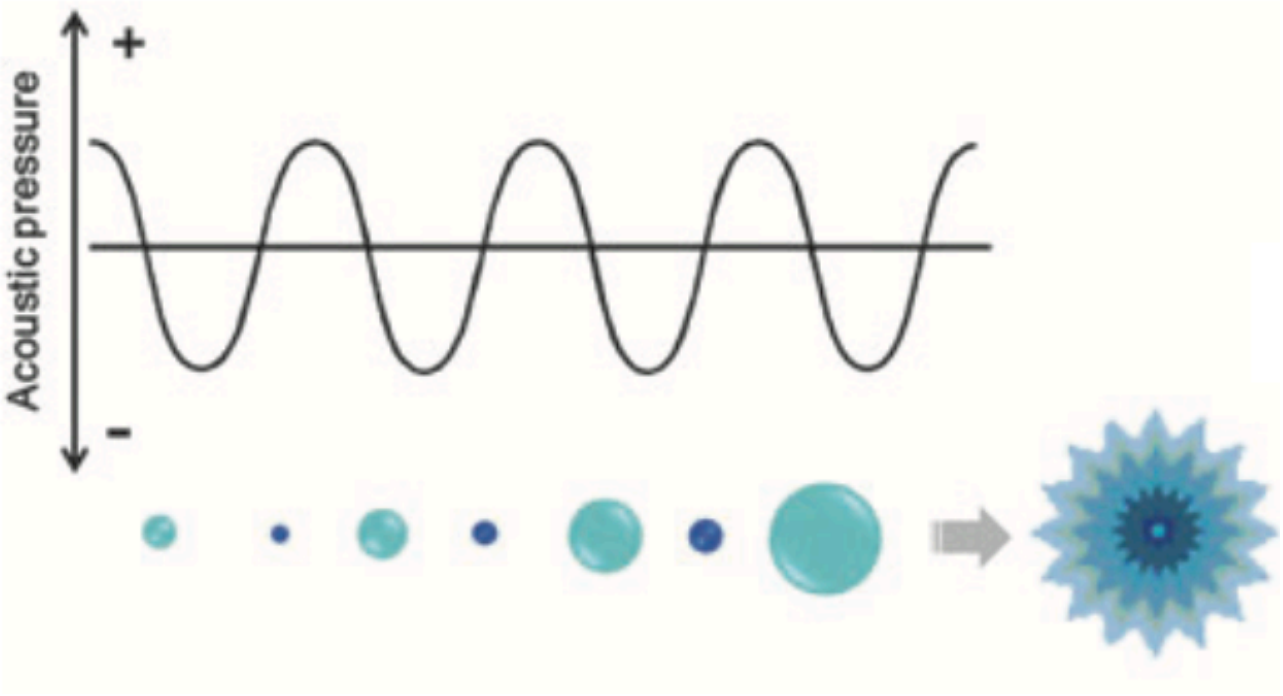


Figure 1 Drawing showing the formation, growth, and collapse of a cavitation bubble.

Cavitation has three stages.

1. Nucleation
2. Bubble growth
3. Implosive collapse

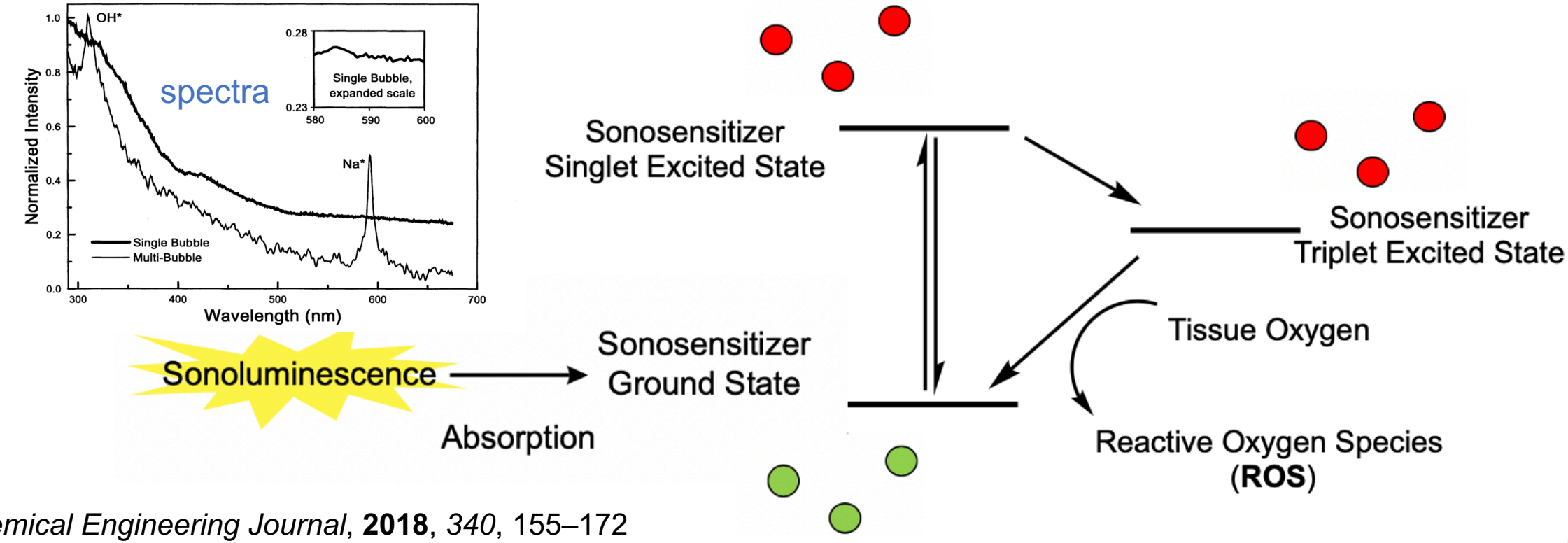
When it collapses, the local temperature and pressure can reach >10,000 K and 800 atm.

Walter de Gruyter, Berlin, Boston, *Nanotechnology Reviews*, **2012**, *1*, 173–182

Possible mechanisms of SDT

2. Sonoluminescence

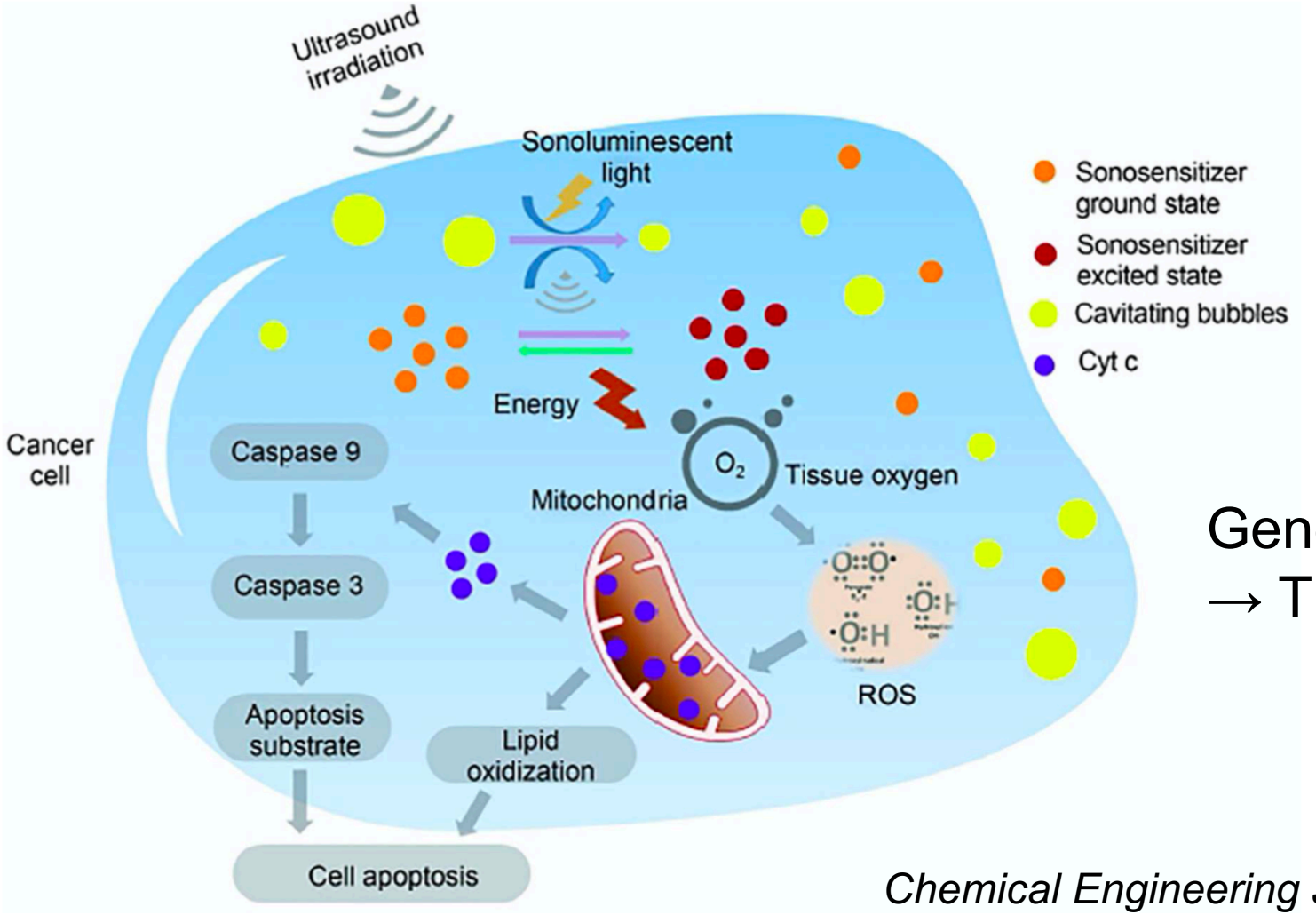
- A concentration of energy caused by cavitation can generates light.
- Sonosensitizers are excited and generates ROS.



Chemical Engineering Journal, **2018**, *340*, 155–172
Thomas J Matula *et al. Phy. Rev. Lett.*, **1995**, *75*, 2602–2605

Possible mechanisms of SDT

3. Cellular toxicity by reactive oxygen species (ROS)



Generated ROS induces apoptosis
→ Therapeutic effect

Chemical Engineering Journal, **2018**, *340*, 155–172

Possible mechanisms of SDT

4. Other Effects

- Pyrolysis of H_2O

  Cavitation also induces pyrolysis of H_2O and generates ROS.

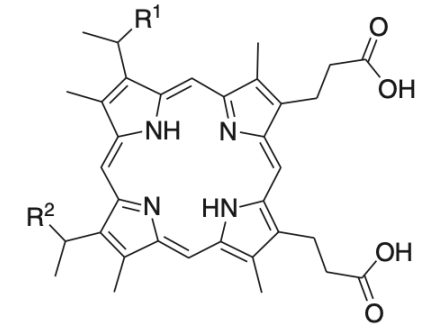
- Mechanical damage

  The strong shock waves are generated by cavitation, resulting in mechanical damage to the cell.

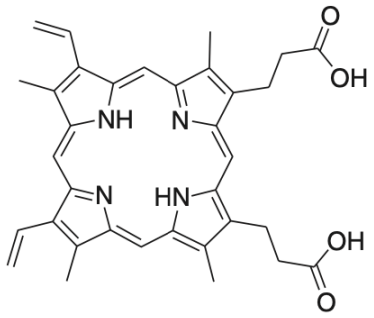
- Thermal Effect

  The transformation and absorption of US energy cause hyperthermia and induce necrosis.

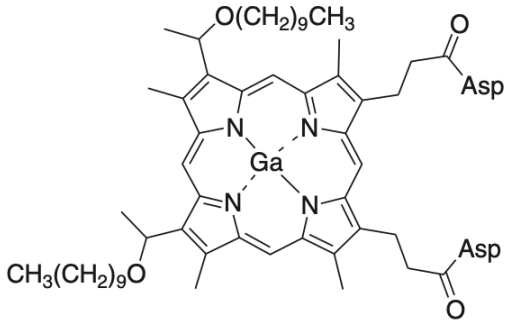
Sonosensitizer



HMME: R^1, R^2 = OCH_3, OH or OH, OCH_3

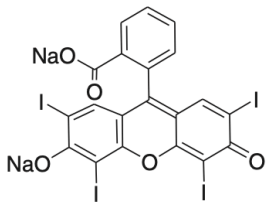


Protoporphyrin IX (PpIX)

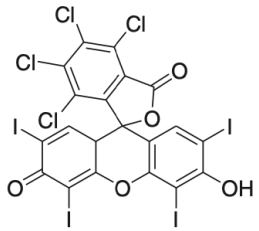


ATX-70

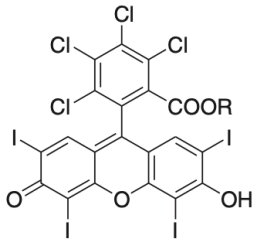
Porphyrins



Erythrosin B (EB)



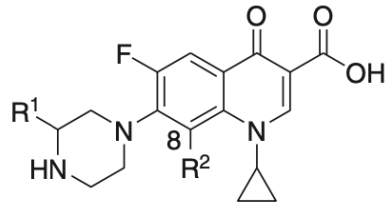
Rose bengal (RB)



RBD2 R=$(CH_2)_7COOH$

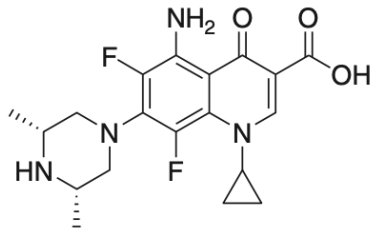
RBD3 R=$CHCOOH(CH_2)_{11}CH_3$

Xanthenes



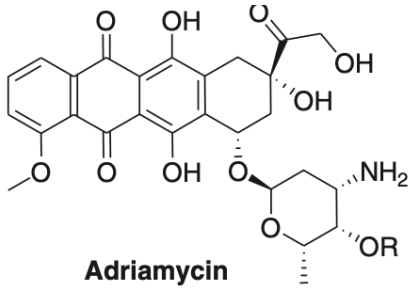
Ciprofloxacin (CPFX): R1 = H, R2 = H

Gatifloxacin (GFLX): R1 = Me, R2 = OMe

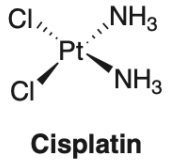


Sparfloxacin (SPFX)

New Quinolones

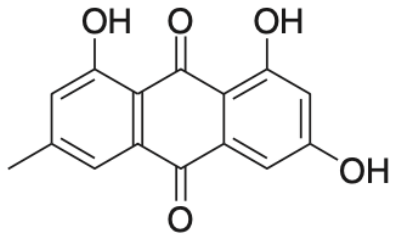


Adriamycin

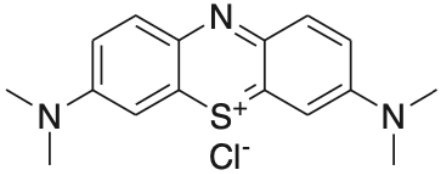


Cisplatin

Antitumor drugs



Emodin



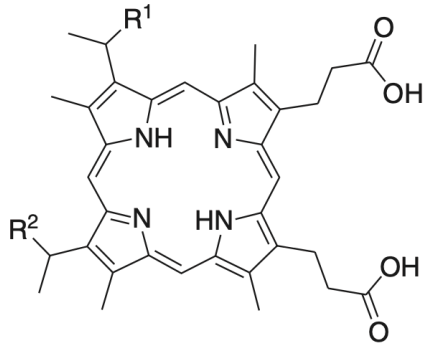
Methylene blue (MB)

Other types

Drug Discovery Today, **2014**, *19*, 504–506

Example of sonosensitizer

sonosensitizer: hematoporphyrin monomethyl ether (HMME)



HMME: R^1, R^2 = OCH_3, OH or OH, OCH_3

Condition
Frequency: 1MHz
Intensity: 1W/cm^2
Time: 60s
[HMME] = 10μg/mL

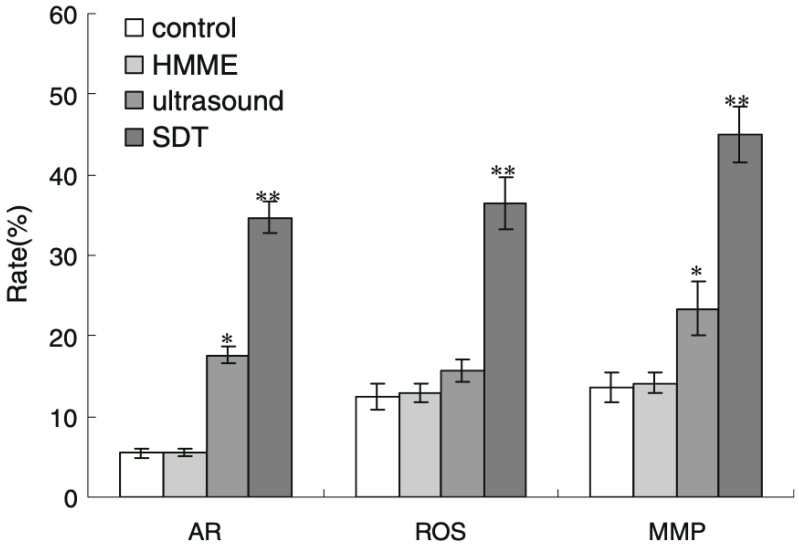


Fig. 2 Apoptotic rate (AR), ROS, and mitochondria membrane potential (MMP, means decreased MMP) of the C6 glioma cells after SDT, ultrasound, HMME, and no treatment by FCM.

ROS generation by HMME+US

→ Mitochondrial damage

→ Induction of apoptosis

→Therapeutic effect for C6 glioma cells

SDT can be a valid way to treat cancer.

S. Dai, S. Hu, C. Wu, *Acta Neurochir.,* **2009**, *151*, 1655 –1661.

Problems of traditional sonosensitizers

Because most of sonosensitizers are strongly hydrophobic, there are some problems as below.

- Low bioavailability
- Fast elimination out of the body
- Poor accumulation into targeted tissue

These problems hinder their clinical translation.

Xiaoqin Qian *et al. Adv. Mater.,* **2016**, *28*, 8097–8129

SDT augmented by nanoparticles (NPs)

Merits

✓ Large surface area suitable for chemical modification

→ Improvement of biocompatibility, biodistribution and selectivity

✓ Combination of SDT with other therapeutic modalities

✓ Decrease of cavitation threshold by NPs

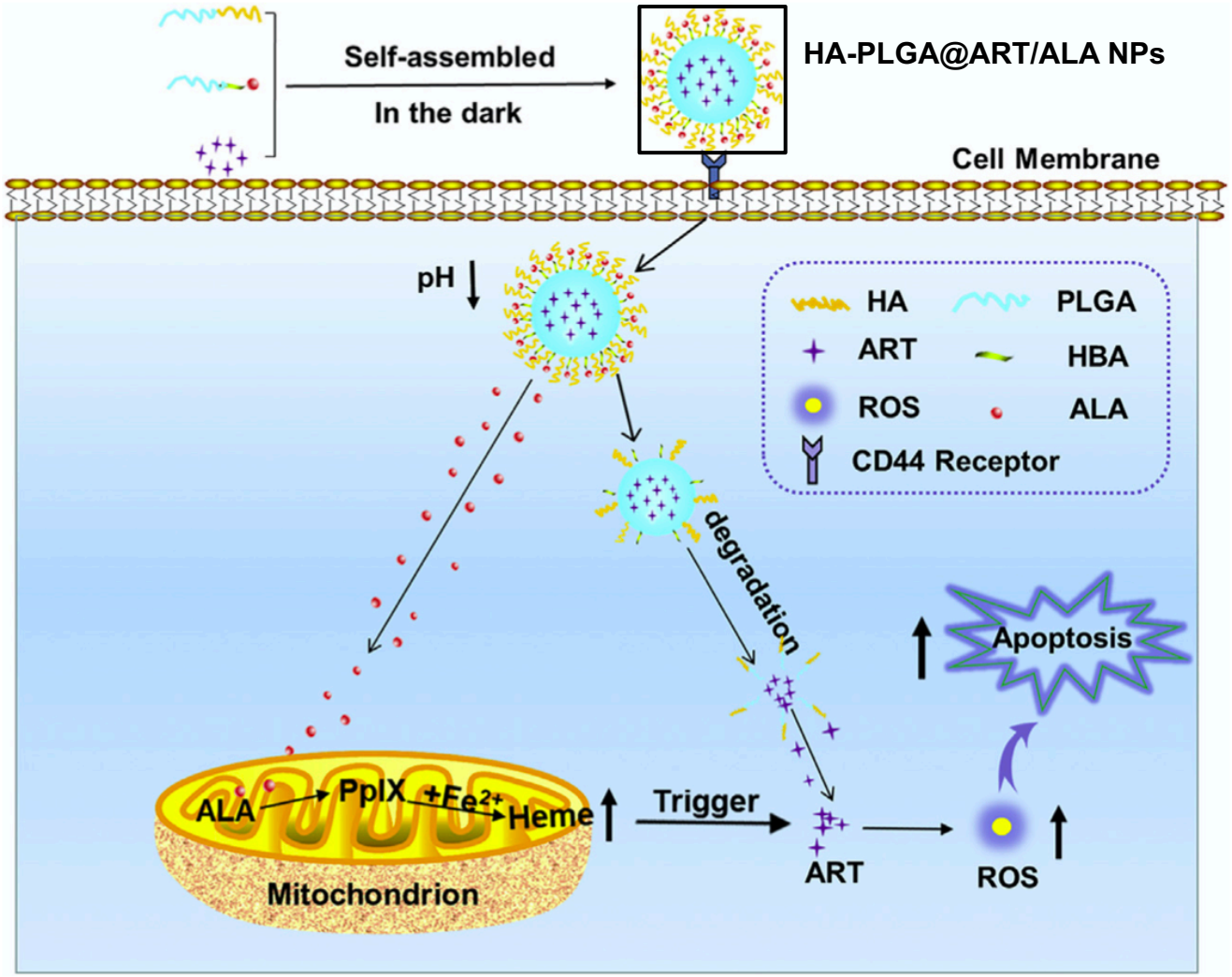
→ Stabilization of nanobubbles on their surface and inside cavities

→ Enhancement of the therapeutic efficiency

Xiaoqin Qian *et al. Adv. Mater.,* **2016**, *28*, 8097–8129

Contents

Self-assembled organic NPs



- Poly(lactic-*co*-glycolic acid) (**PLGA**):

  great biocompatibility and biodegradability
- Hyaluronic acid (**HA**):

  tumor-targeting
- 4-hydrazinobenzoic acid(**HBA**):

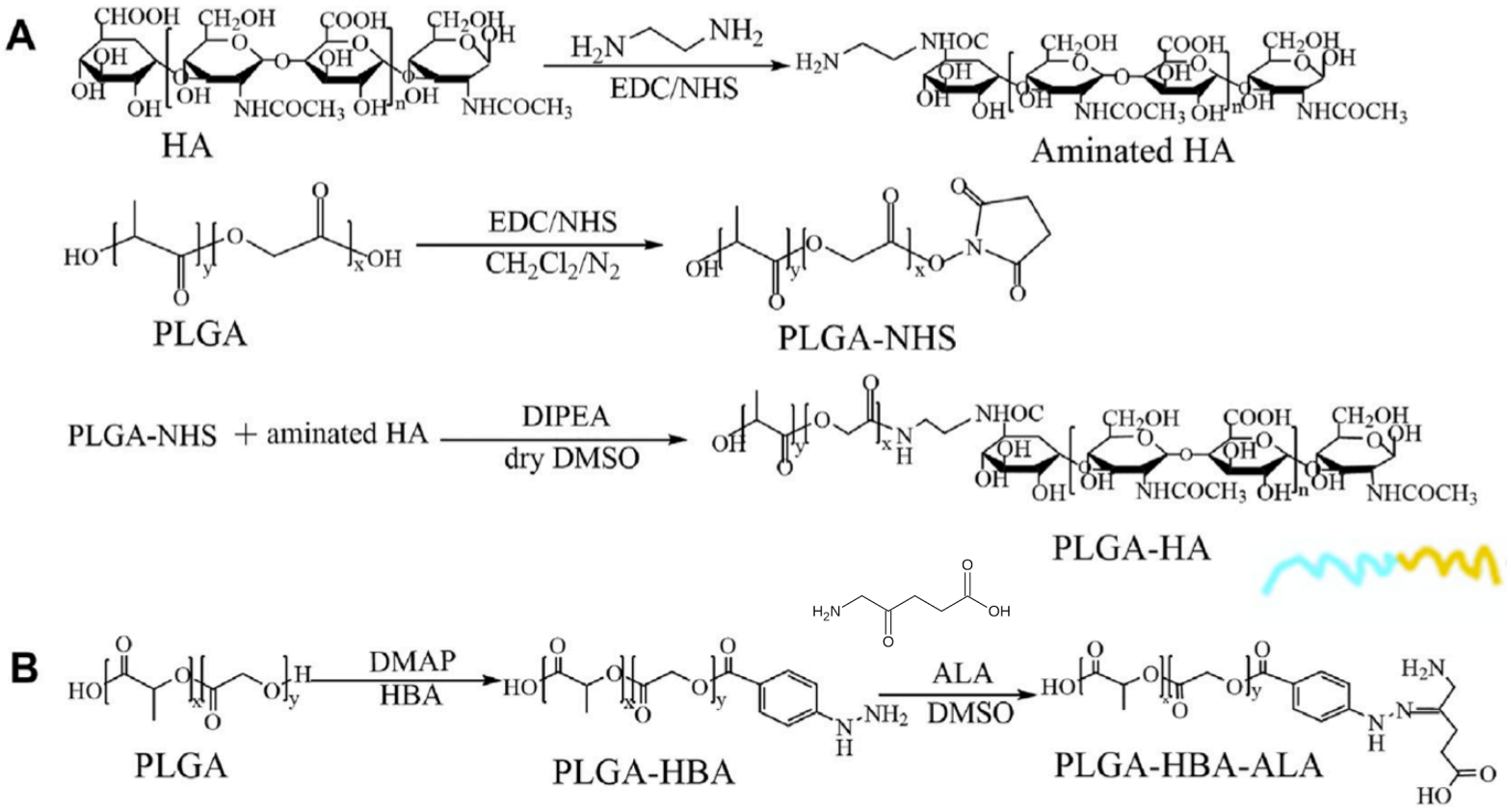
  pH-sensitive linkage
- Artemisinin (**ART**):

  ROS generation (as a sonosensitizer)
- Amino levulinic acid (**ALA**):

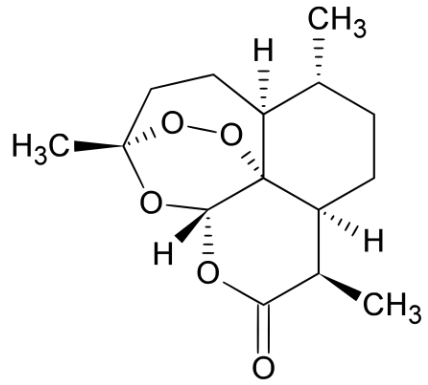
  precursor of Heme and activate ART (also as a sonosensitizer)

Lei Wang, *et al. J. Controlled Release*, **2018**, *286*, 74–84.

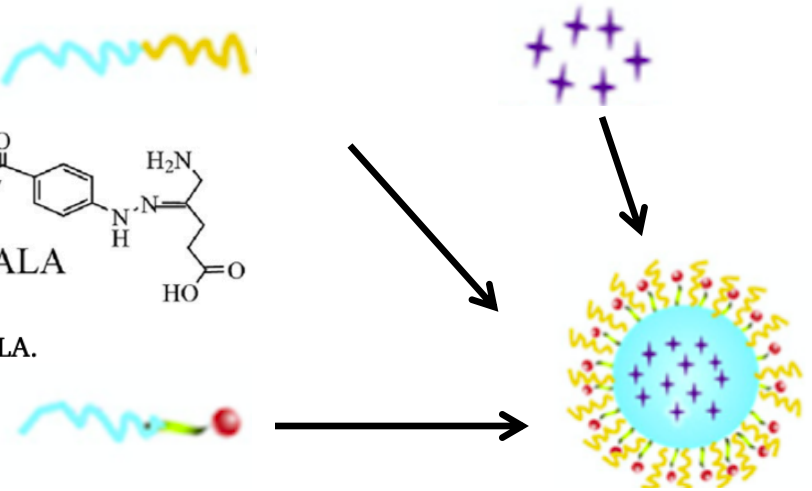
Structure of HA-PLGA@ART/ALA NPs



Scheme 2. Synthesis schemes of the block copolymers. (A) PLGA-HA, (B) PLGA-HBA-ALA.



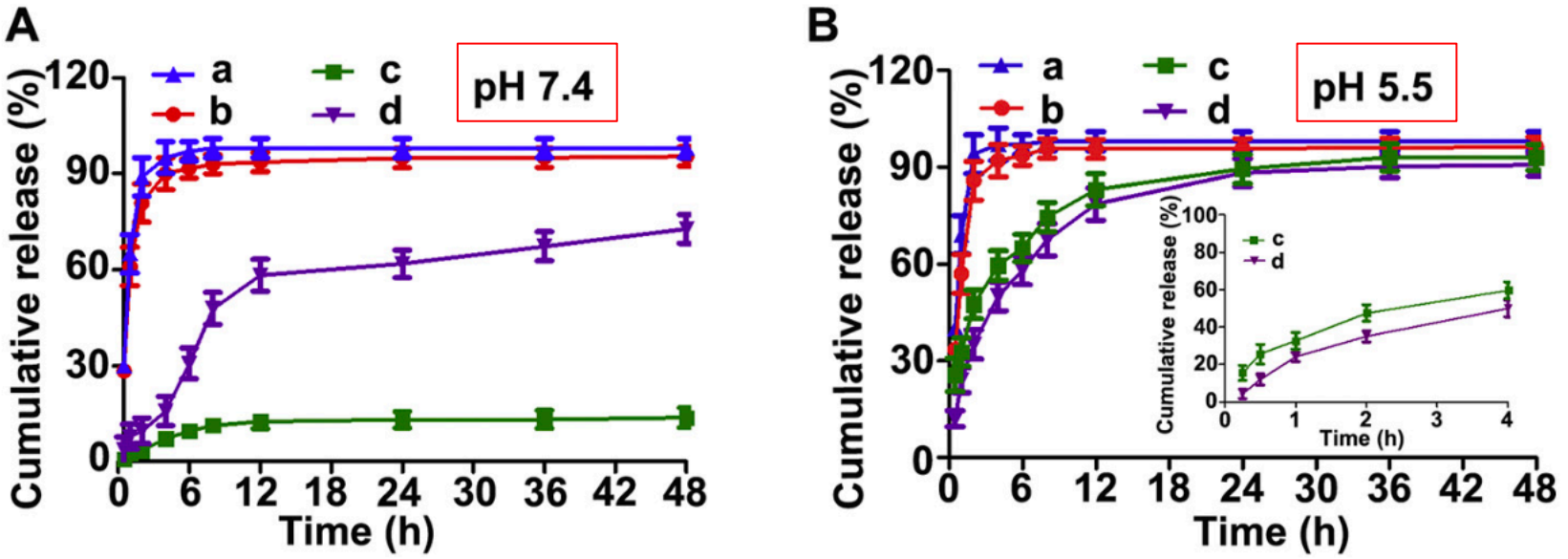
Artemisinin



NP were prepared by self-assembled method.
Hydrophobic ART was encapsulated in the inner core.

Lei Wang, *et al. J. Controlled Release*, **2018**, *286*, 74–84.

In vitro pH-sensitive drug release assay



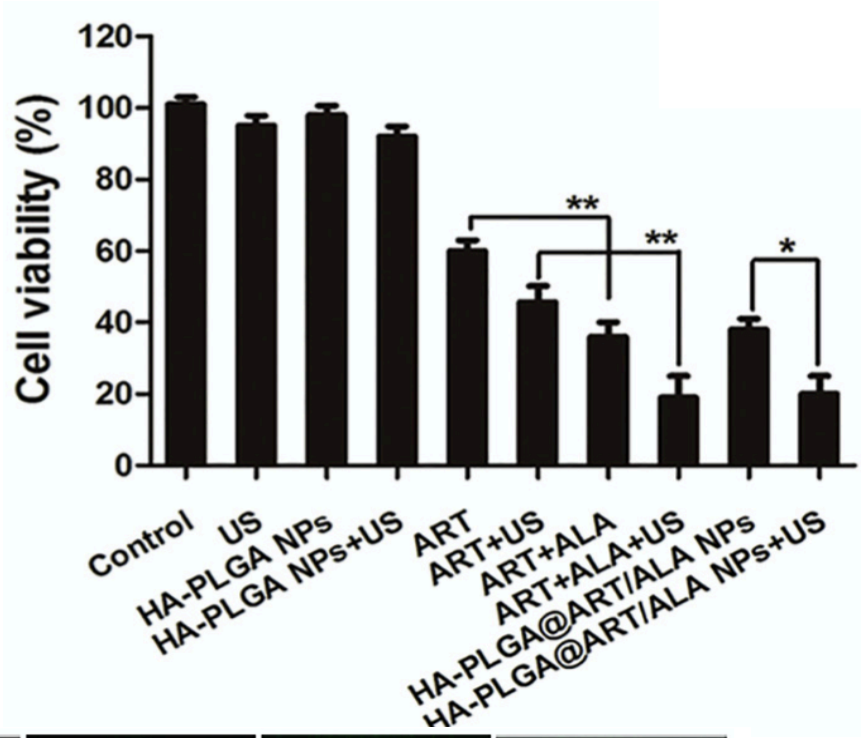
a. free ALA
b. free ART
c. ALA from NPs
d. ART from NPs

- ALA were released under the acidic condition.
- At pH 5.5, ALA was released faster than ART, especially at the early time points.

Lei Wang, *et al. J. Controlled Release*, **2018**, *286*, 74–84.

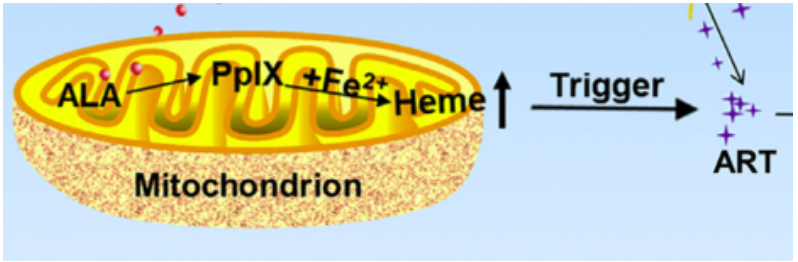
Cytotoxicity and ROS production

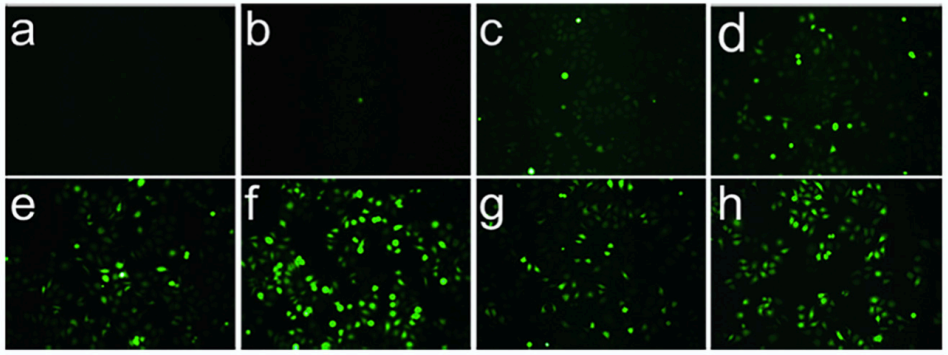
Condition
Intensity: 2 W/cm^2
Time: 60 s
[ART] = 88 μM
[ALA] = 0.5 mM



* More ROS generation by US

** Combination of ALA & ART

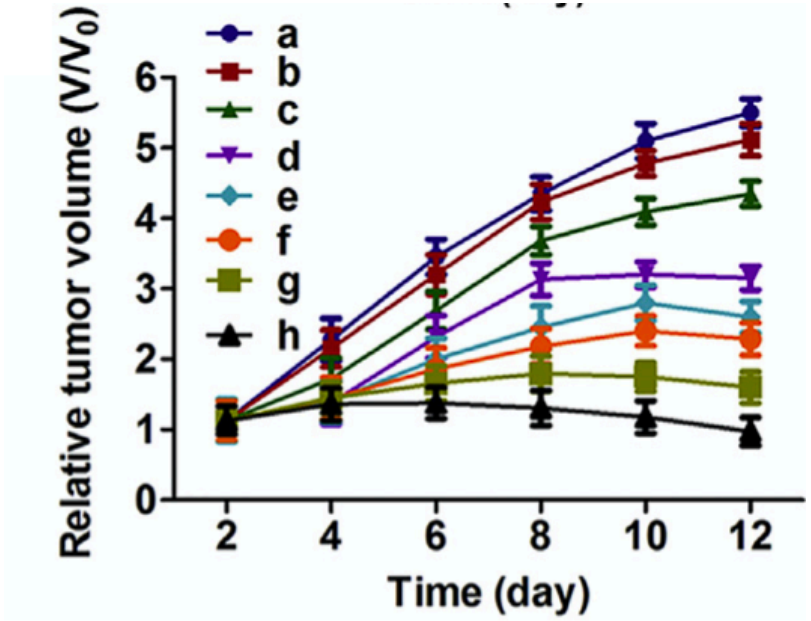


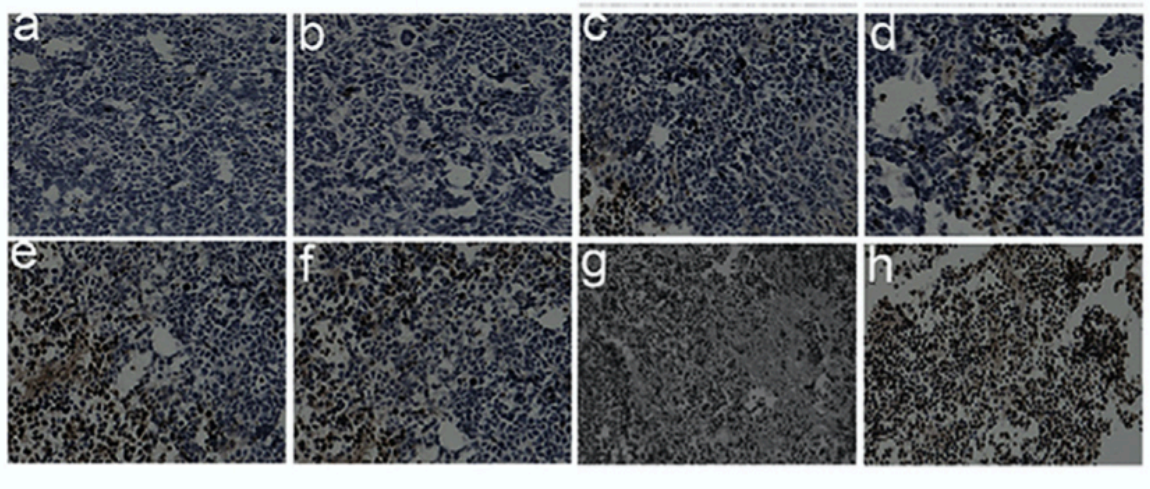


a. Control	e. ART+ALA
b. US	f. ART+ALA+US
c. ART	g. HA-PLGA@ART/ALA NPs
d. ART+US	h. HA-PLGA@ART/ALANPs NPs + US

Lei Wang, *et al. J. Controlled Release*, **2018**, *286*, 74–84.

Animal experiments





NPs + US group was the most effective.

- ✓ More drug accumulation by HA
- ✓ ART & ALA combination
- ✓ More ROS production by US

a. Control	e. ART+ALA
b. US	f. ART+ALA+US
c. ART	g. HA-PLGA@ART/ALA NPs
d. ART+US	h. HA-PLGA@ART/ALANPs NPs + US

Lei Wang, *et al. J. Controlled Release*, **2018**, *286*, 74–84.

Animal experiments

○: Tumor site

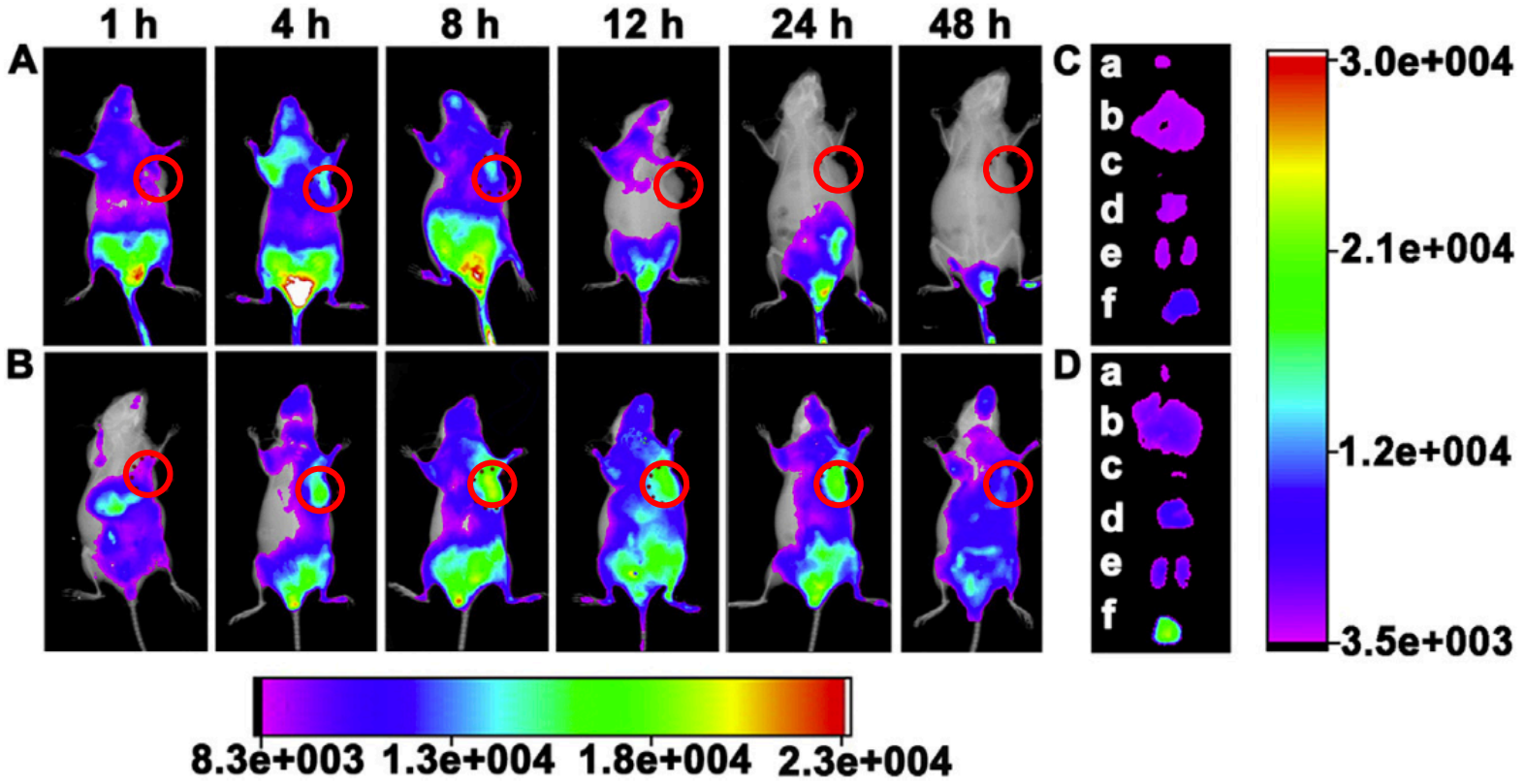


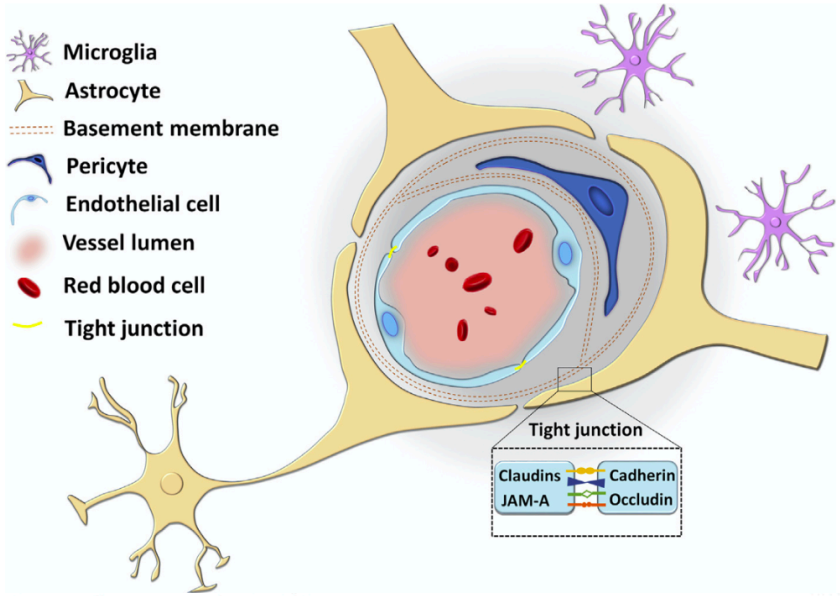
Fig. 7. *In vivo* NIR fluorescence imaging. Representative *in vivo* fluorescence images of tumor-bearing mice following tail vein administration of (A) IR780 and (B) HA-PLGA@IR780 NPs at different time points. *Ex vivo* NIR fluorescence images of major organs and tumors dissected from mice at 48 h after injection of (C) IR780 and (D) HA-PLGA@IR780 NPs: (a) heart, (b) liver, (c) spleen, (d) lung, (e) kidney, (f) tumor.

- ✓ Tumor targeting by HA
- ✓ Long circulation characteristic

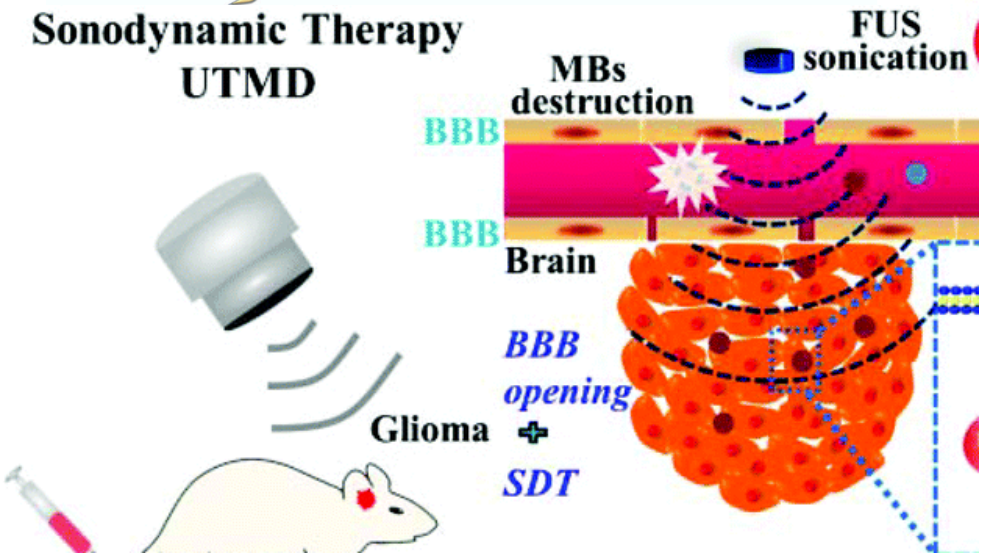
Lei Wang, *et al. J. Controlled Release*, **2018**, *286*, 74–84.

Contents

BBB opening by microbubbles and US



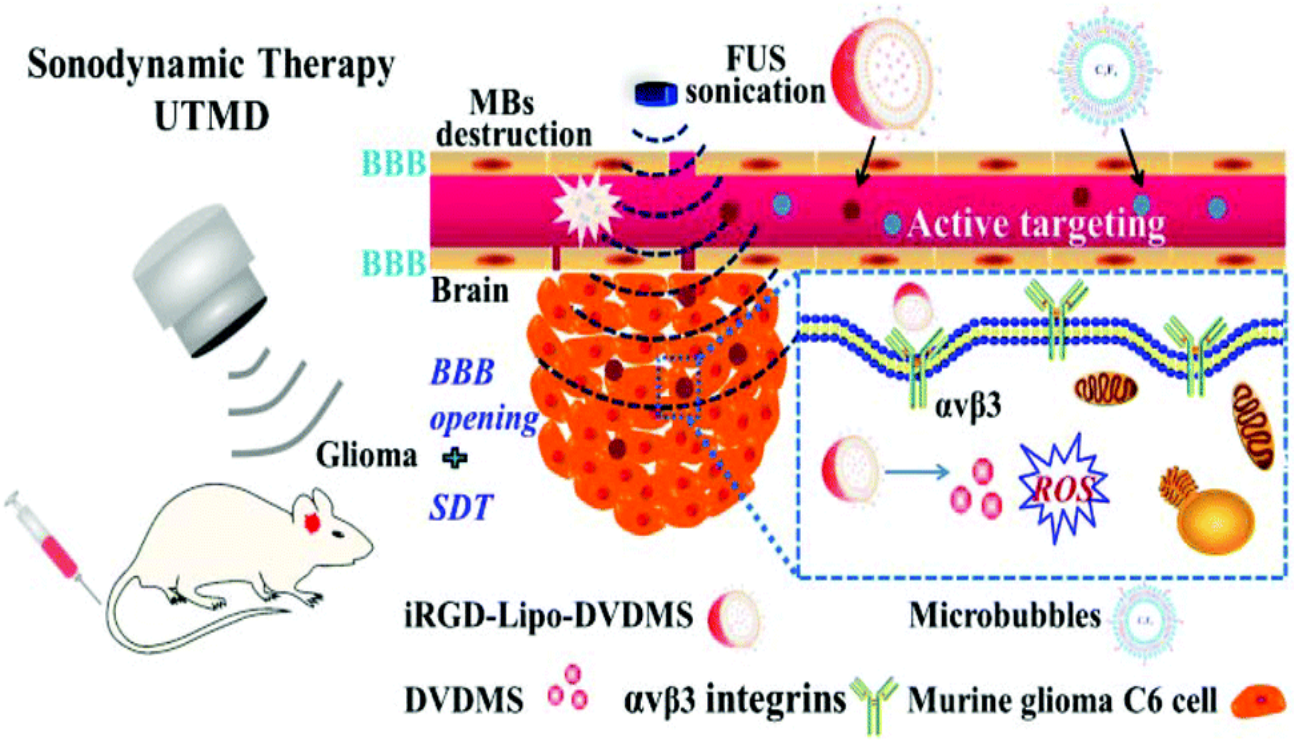
BBB (blood-brain barrier) is the main obstacle to the drug delivery.



BBB can be opened up locally and reversibly by **U**ltrasound-**T**argeted **M**icrobubble **D**estruction (**UTMD**).

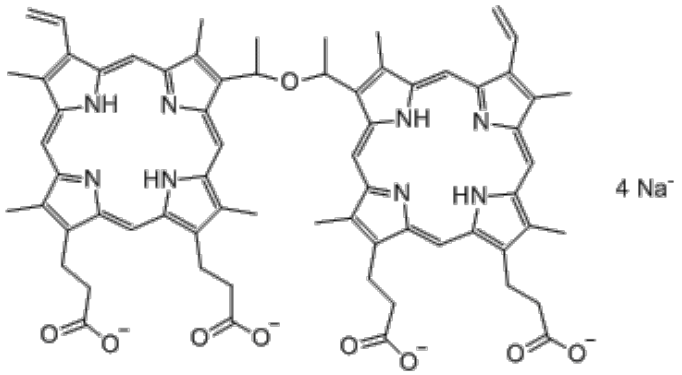
Yue Sun *et al*. *Biomater. Sci.,* **2019**, *7*, 985–994.
Xiaobing Wang *et al. Ultrason. Sonochem.,* **2017**, *37*, 592–599

NPs-assisted SDT for glioma



- Liposome
  Drug carrier
- DVDMS (Sinoporphyrin sodium)
  Hydrophilic sonosensitizer
- *αvβ3* integrin
  Specifically expressed in tumor cells
- iRGD
  αvβ3 integrin-targeting peptide

DVDMS

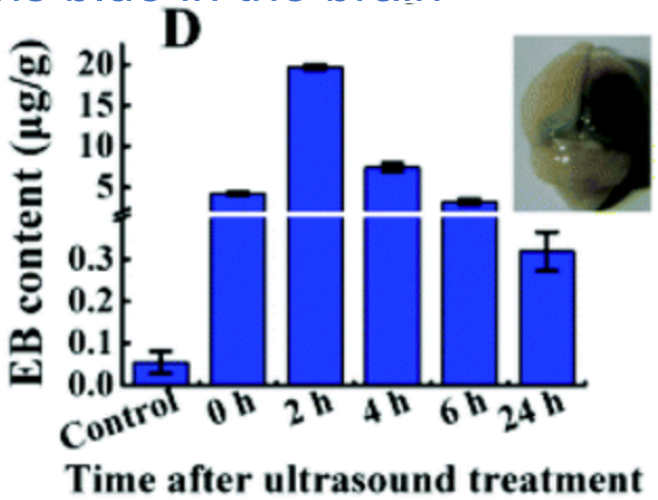


Yue Sun *et al*. *Biomater. Sci.*, **2019**, *7*, 985–994.

BBB opening *in vivo*

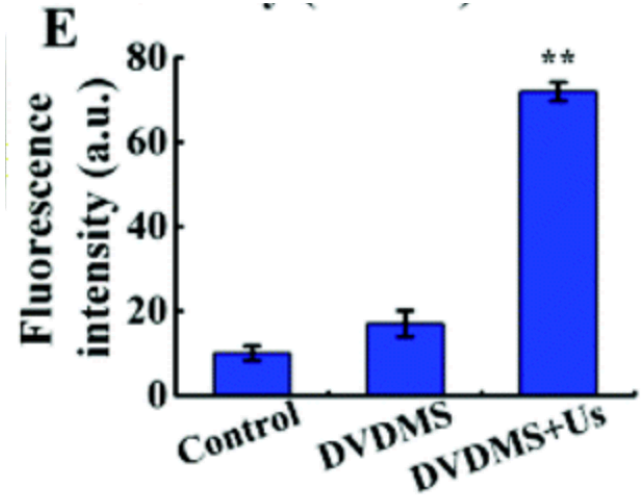
Content of Evans blue in the brain

Condition
Frequency: 1.0 MHz
Power: 1 W
Exposure Time: 1 min
Interval Time: 1 s



BBB showed the highest permeability 2h after UTMD.

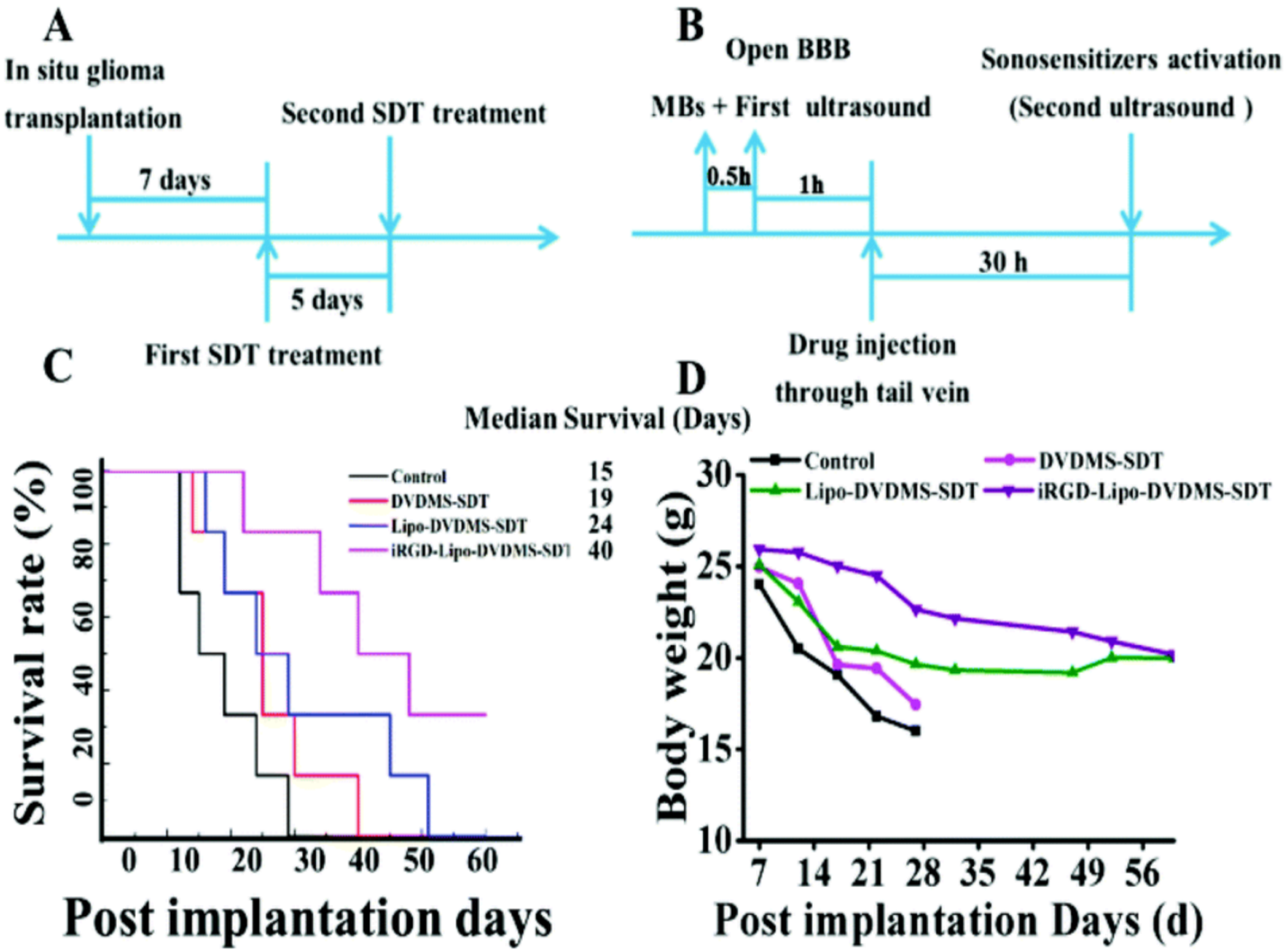
DVDMS accumulation in orthotopic gliomas



The penetration of DVDMS with UTMD was 7.2-fold higher than that without UTMD.

Yue Sun *et al*. *Biomater. Sci.*, **2019**, *7*, 985–994.

Sonodynamic effect *in vivo*



Condition(UTMD)
Frequency: 1.0 MHz
Power: 1 W
Exposure Time: 1 min
Interval Time: 1 s

Condition (SDT)
Frequency: 1.0 MHz
Power: 1 W
Exposure Time: 1 min

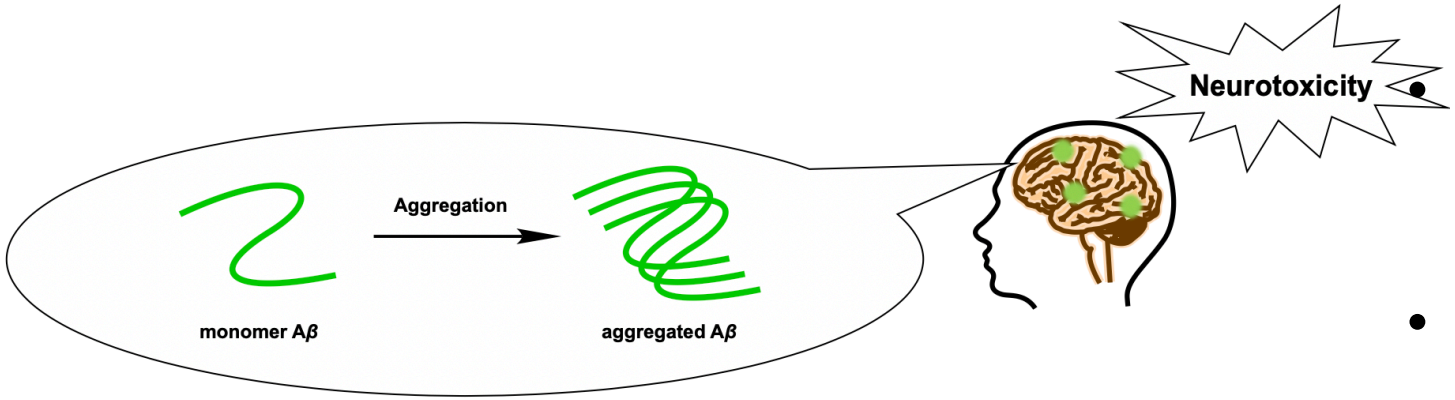
Survival time of mice treated with iRGD-Lipo-DVDMS-SDT was longer than the others.

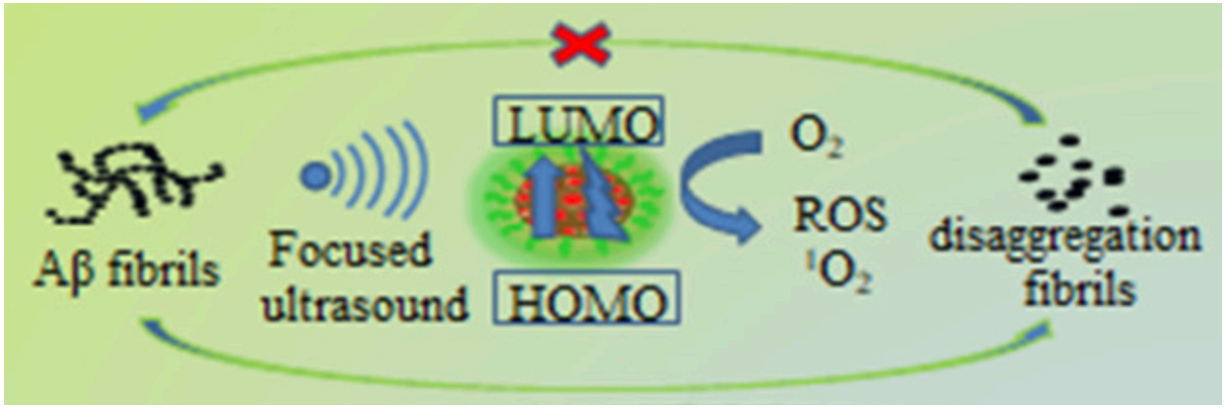
- ✓BBB opening
- ✓tumor targeting by iRGD
- ✓DVDMS delivery by liposome

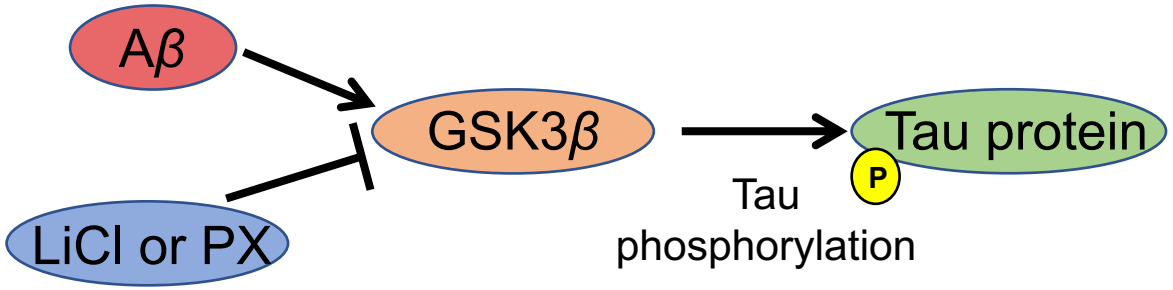
Yue Sun *et al*. *Biomater. Sci.,* **2019**, *7*, 985–994.

Contents

Alzheimer disease



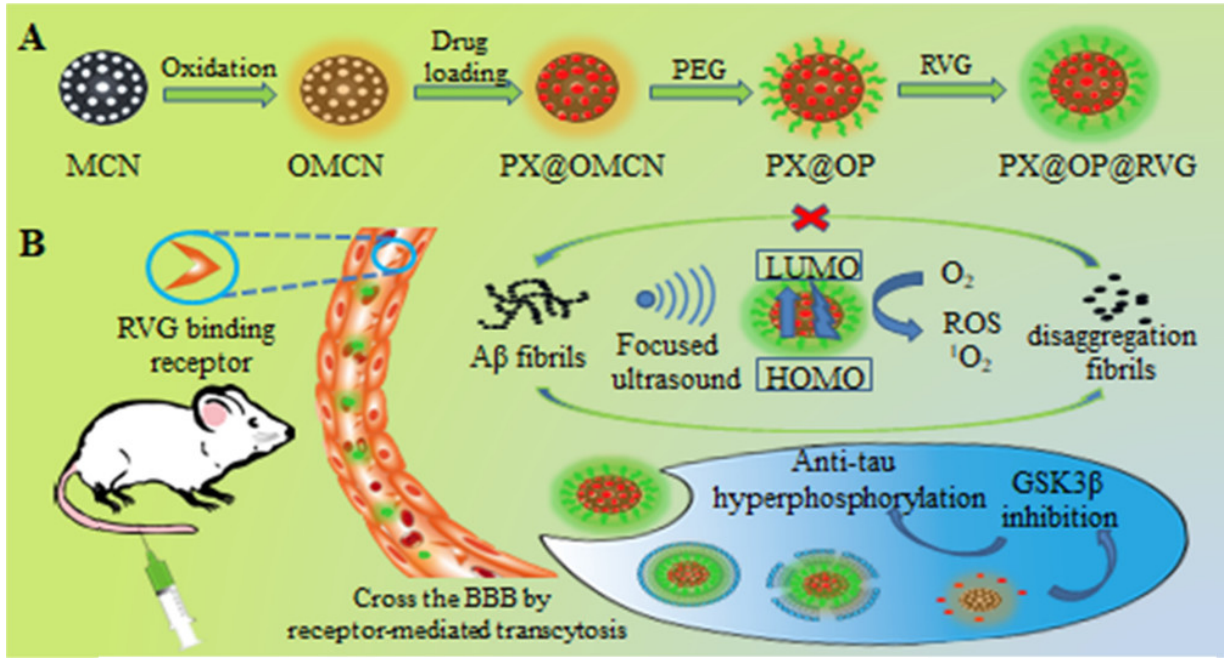


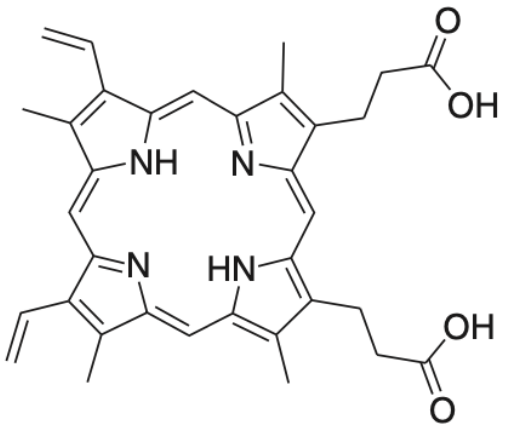


- Aggregation of Aβ is main cause of Alzheimer disease (AD).
- Oxygenation of Aβ by sonosensitizer with US may be a valid way to treat AD.
- Tau hyperphosphorylation is also a cause of AD.
- Inhibiting Glycoprotein synthase kinase-3 β (GSK3β) is a hopeful treatment.

Mengmeng Xu *et al*. *ACS Appl. Mater. Interfaces*, **2018**, *10*, 32965–32980

IX-modified multifunctional NPs





Protoporphyrin IX (PpIX)

Sonosensitizer

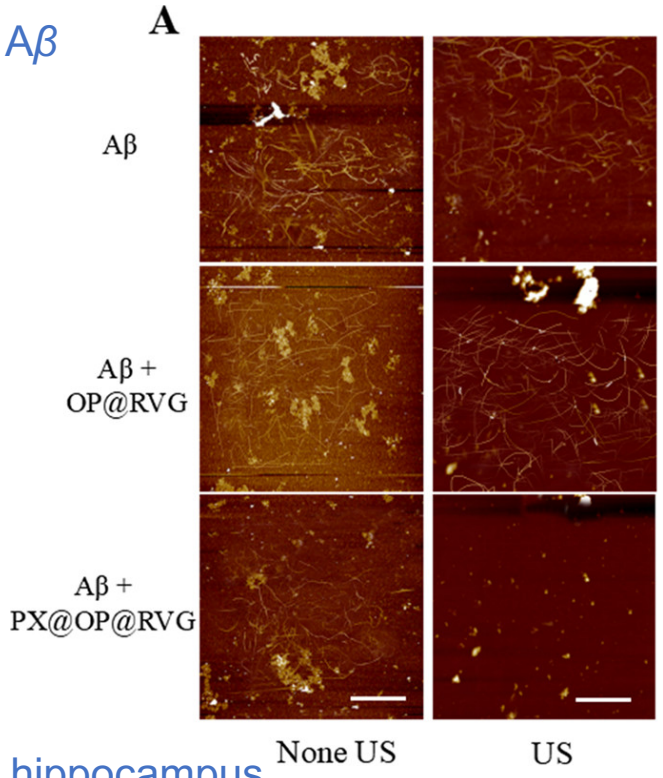
- Oxidized mesoporous carbon nanosphere (**OMCN**): drug carrier
- PpIX or **PX**: sonosensitizer and GSK3*β* inhibitor
- Rabies Virus Glycoprotein (**RVG**): It improves BBB penetration and selectivity of brain cell

Mengmeng Xu *et al*. *ACS Appl. Mater. Interfaces*, **2018**, *10*, 32965–32980

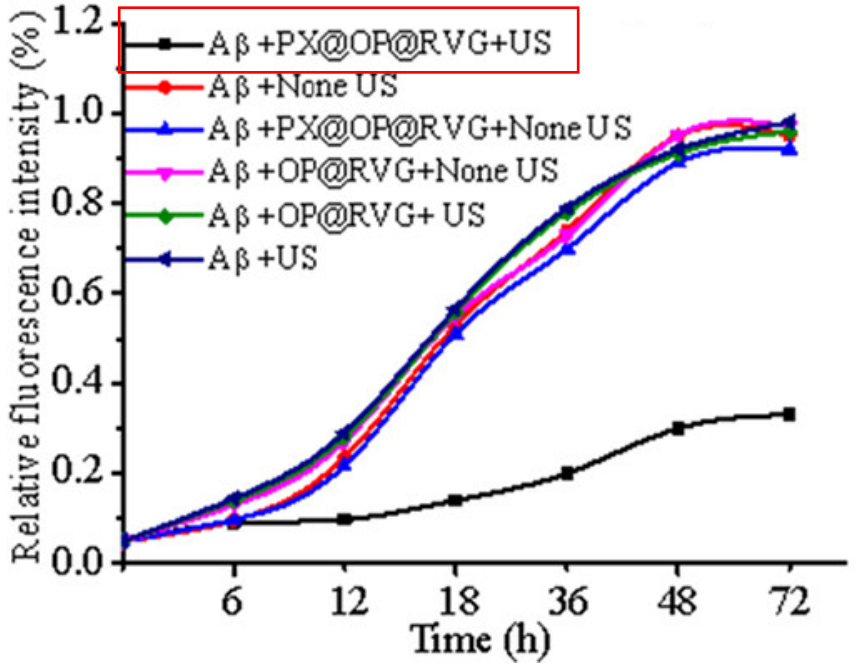
Inhibition of Aβ aggregation

AFM image of Aβ

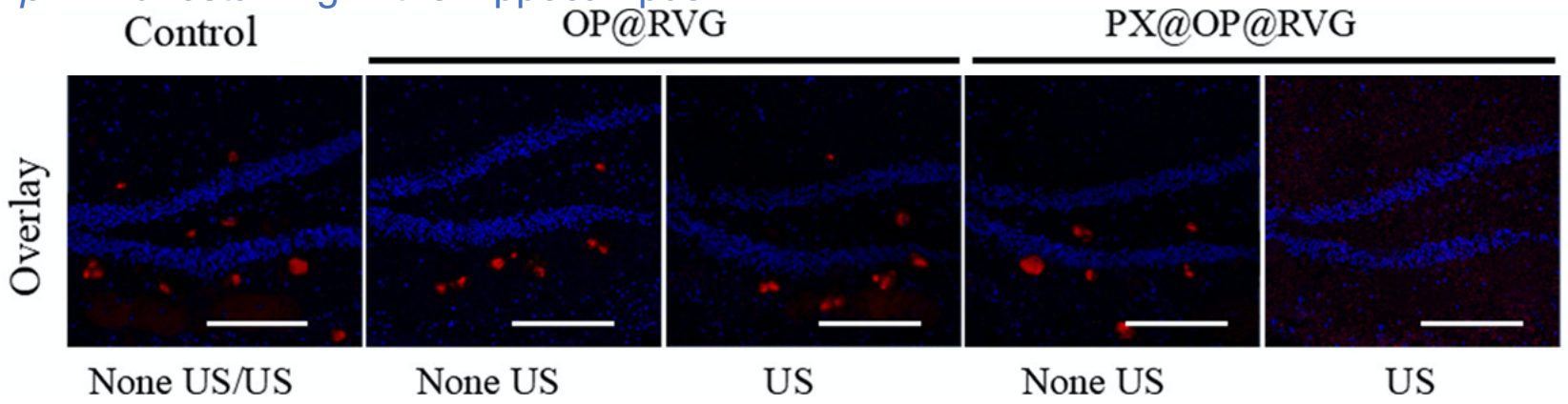
Condition
Frequency: 1 MHz
Intensity: 3 W/cm^2
Time: 3 min
[NPs] = 20 μg/mL



ThT fluorescence assay



Aβ immunostaining in the hippocampus

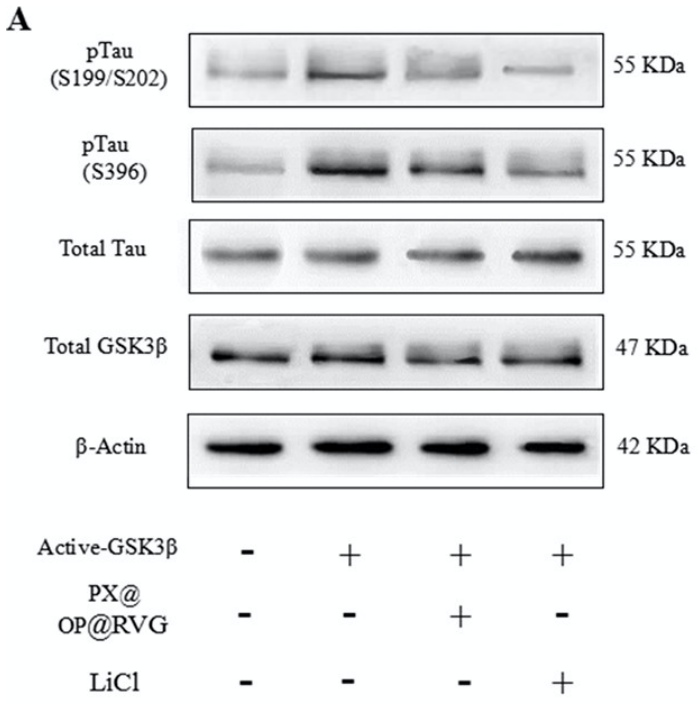


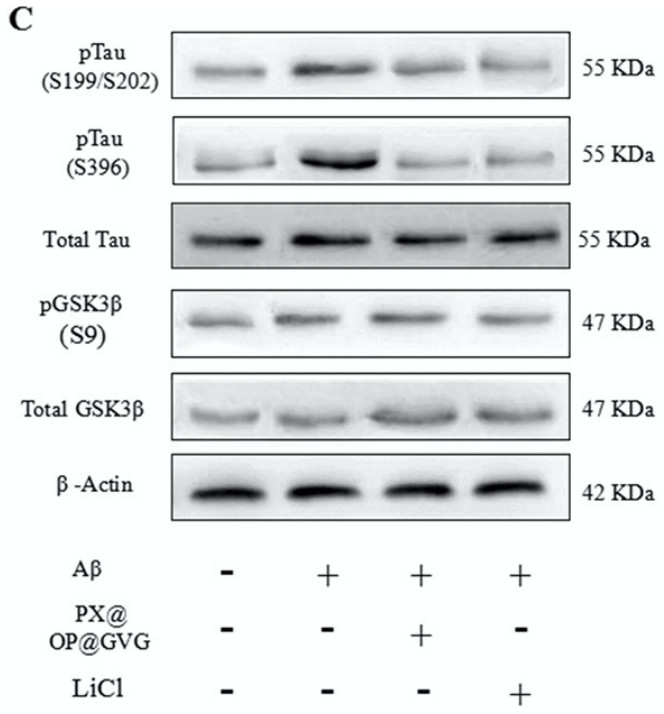
NPs + US remarkably hindered Aβ aggregation.

Mengmeng Xu *et al*.
ACS Appl. Mater. Interfaces, **2018**, *10*, 32965–32980

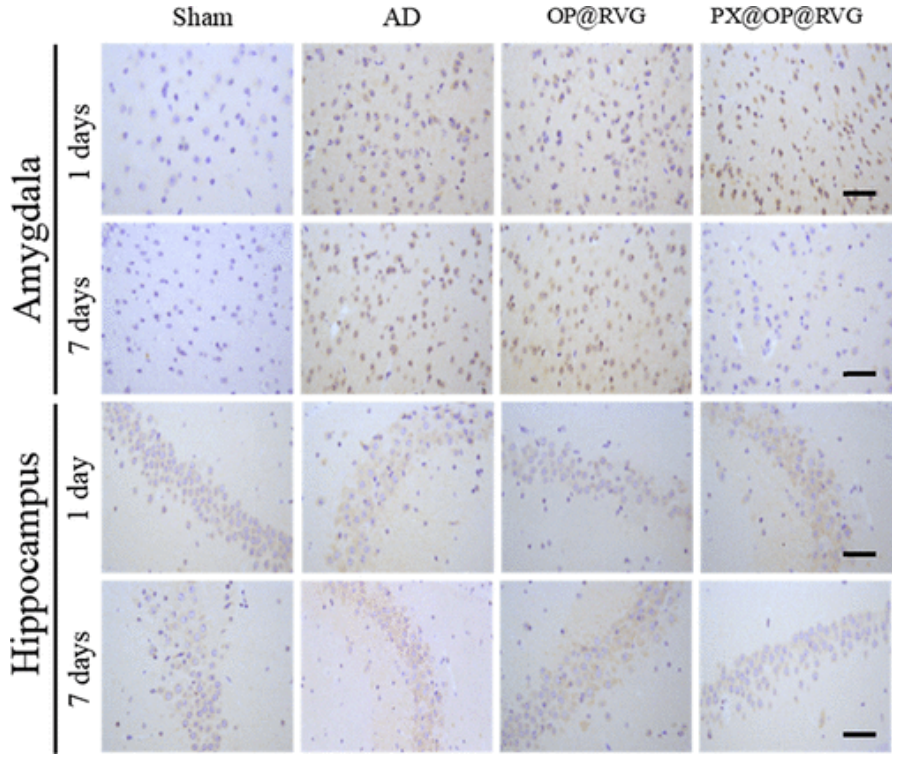
Inhibition of tau hyperphosphorylation

Inhibition of tau phosphorylation assay





NPs effect in AD mice model

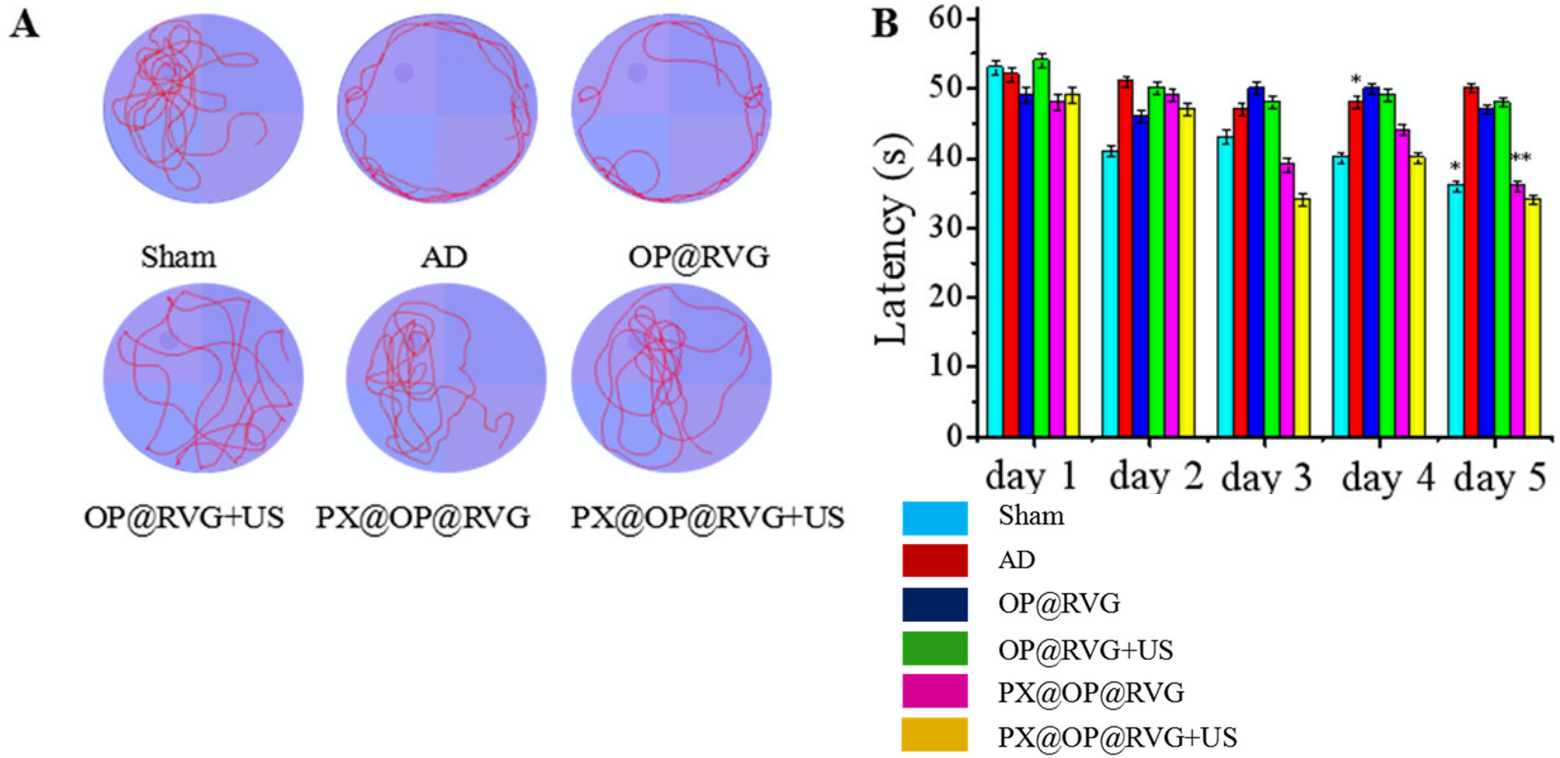


Treatment with NPs downregulated tau hyperphosphorylation.

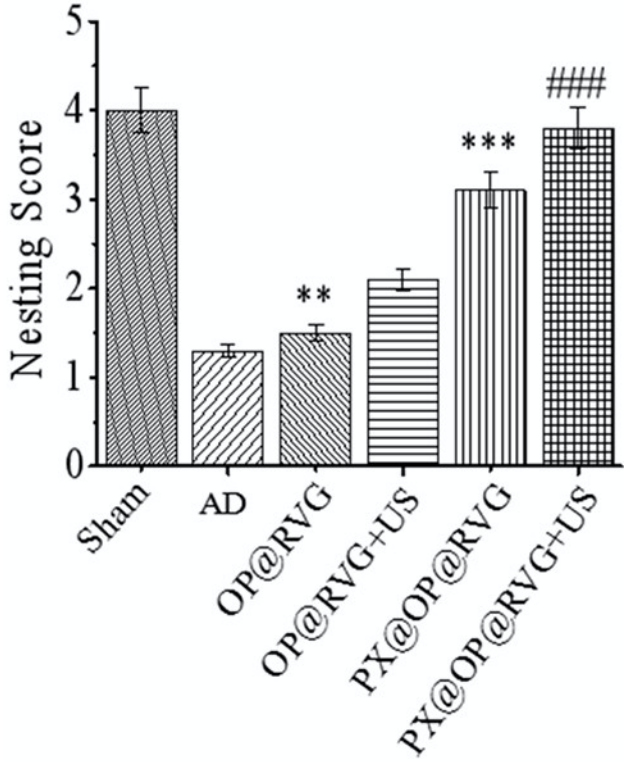
Mengmeng Xu *et al*. *ACS Appl. Mater. Interfaces*, **2018**, *10*, 32965–32980

Rescue of memory deficits

Morris Water Maze test



Nest construction



PX@OP@RVG group showed improved learning ability.

Nest construction test showed similar results.

Mengmeng Xu *et al*. *ACS Appl. Mater. Interfaces*, **2018**, *10*, 32965–32980

Contents

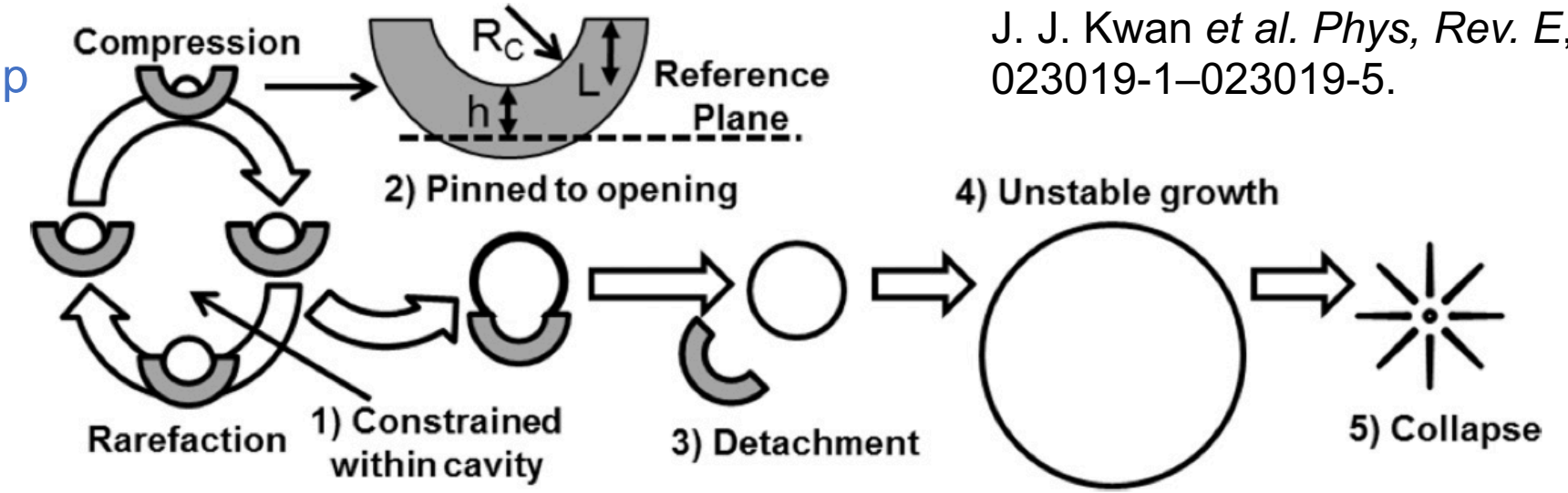
Summary

- SDT is a novel non-invasive therapeutic modality.
- NPs enhances therapeutic effect of SDT.
- BBB opening by UTMD can solve the main problem of drug delivery system.
- NP-augmented SDT can be applied to not only cancer but also Alzheimer disease.

Appendix

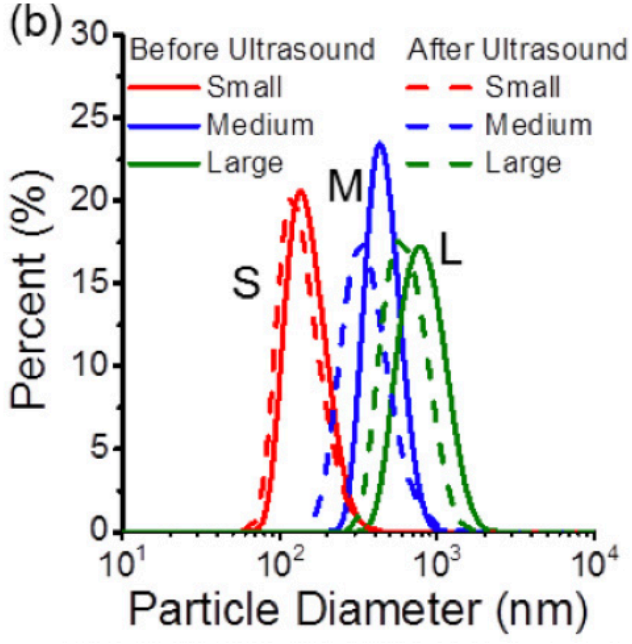
Nanocup decreases cavitation threshold (P12)

Schematic of nanocup

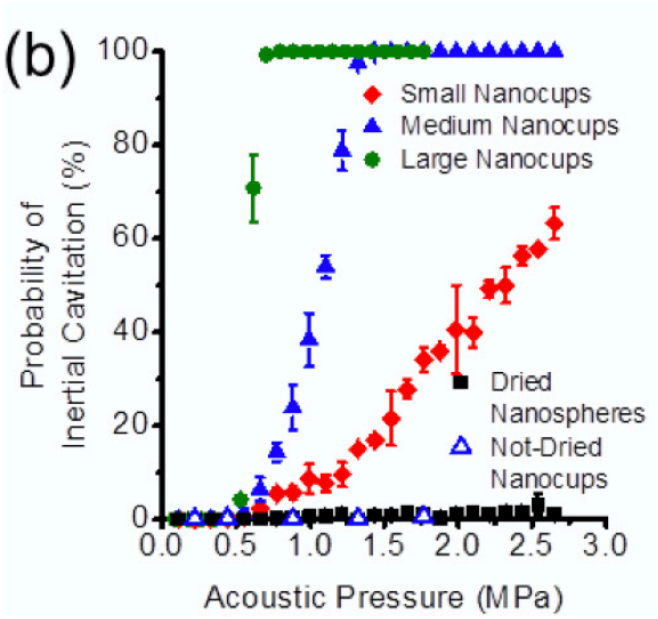


J. J. Kwan *et al. Phys, Rev. E*, **2015**, *92,* 023019-1–023019-5.

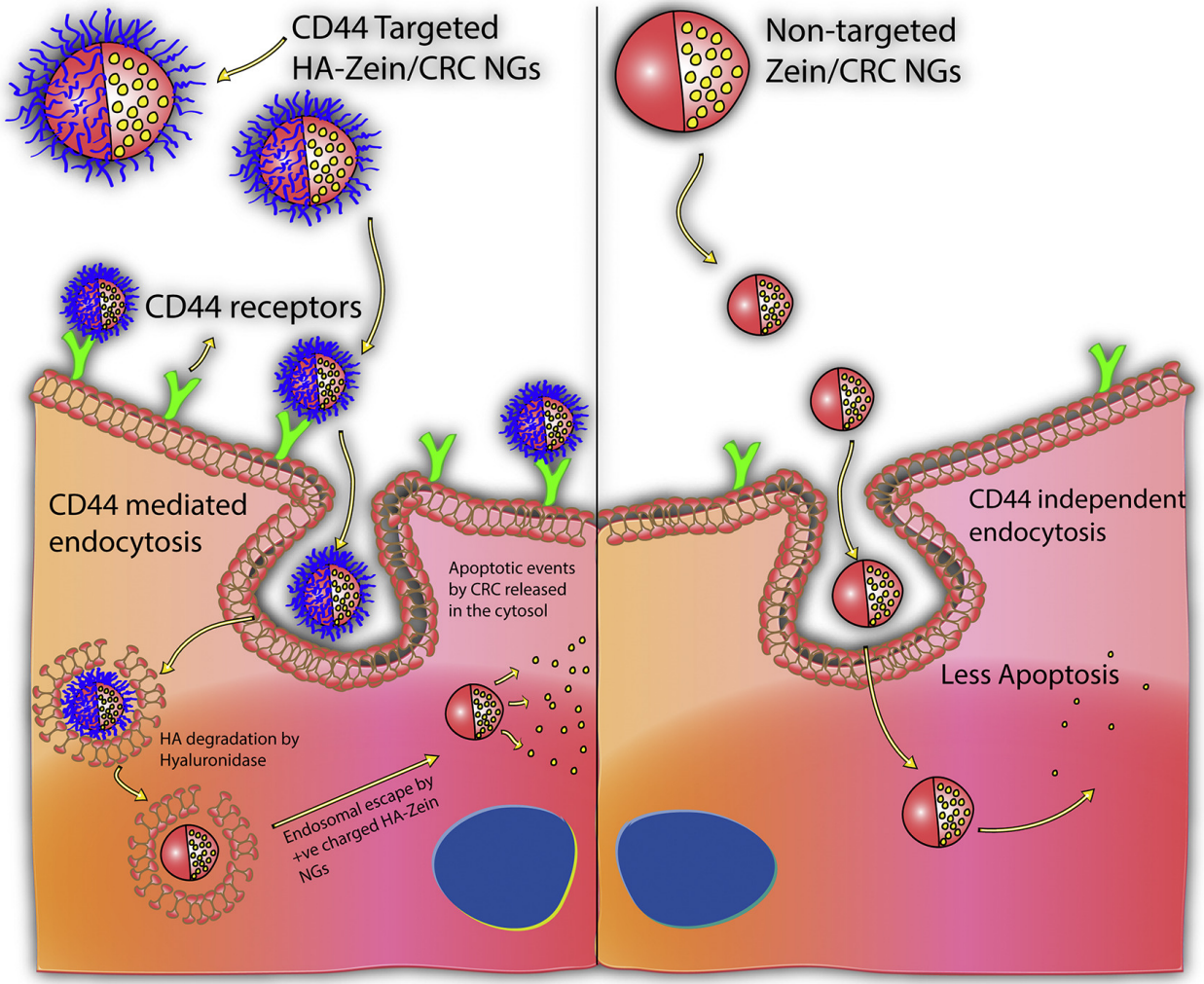
Size measurement



Probability of inertial cavitation curve in each size



CD-44 targeting by hyaluronic acid (HA)

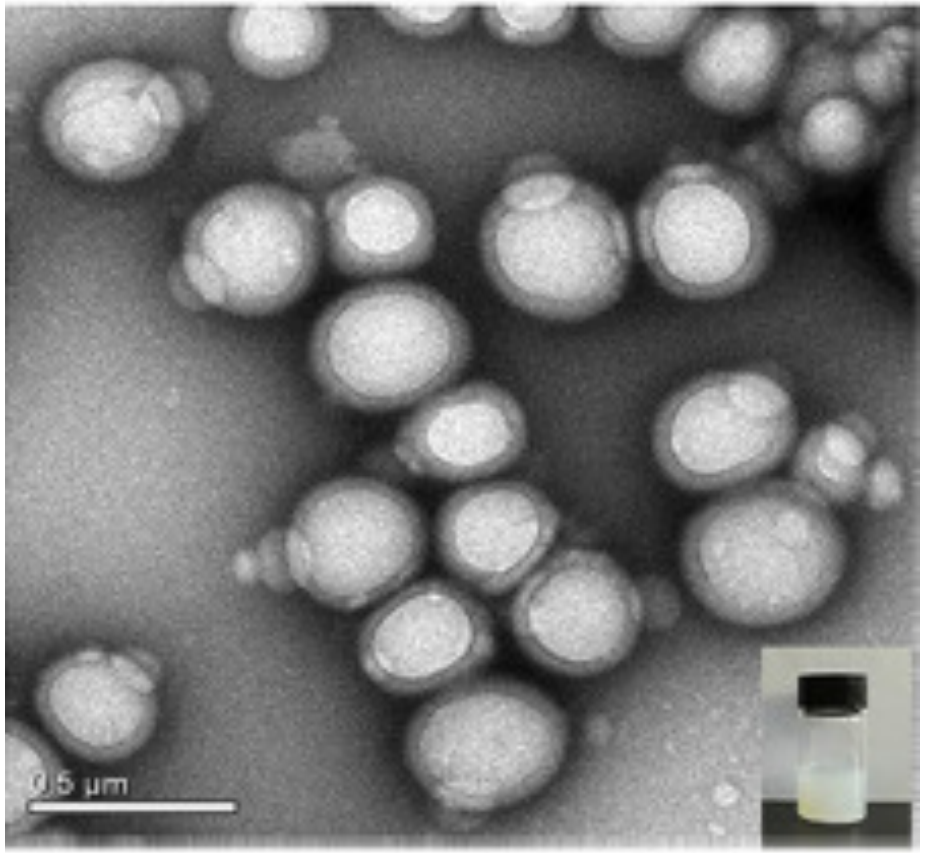


- HA recognize CD-44 receptors, which are overexpressed in tumor cells.
- NPs are then internalized via CD-44 mediated endocytosis.

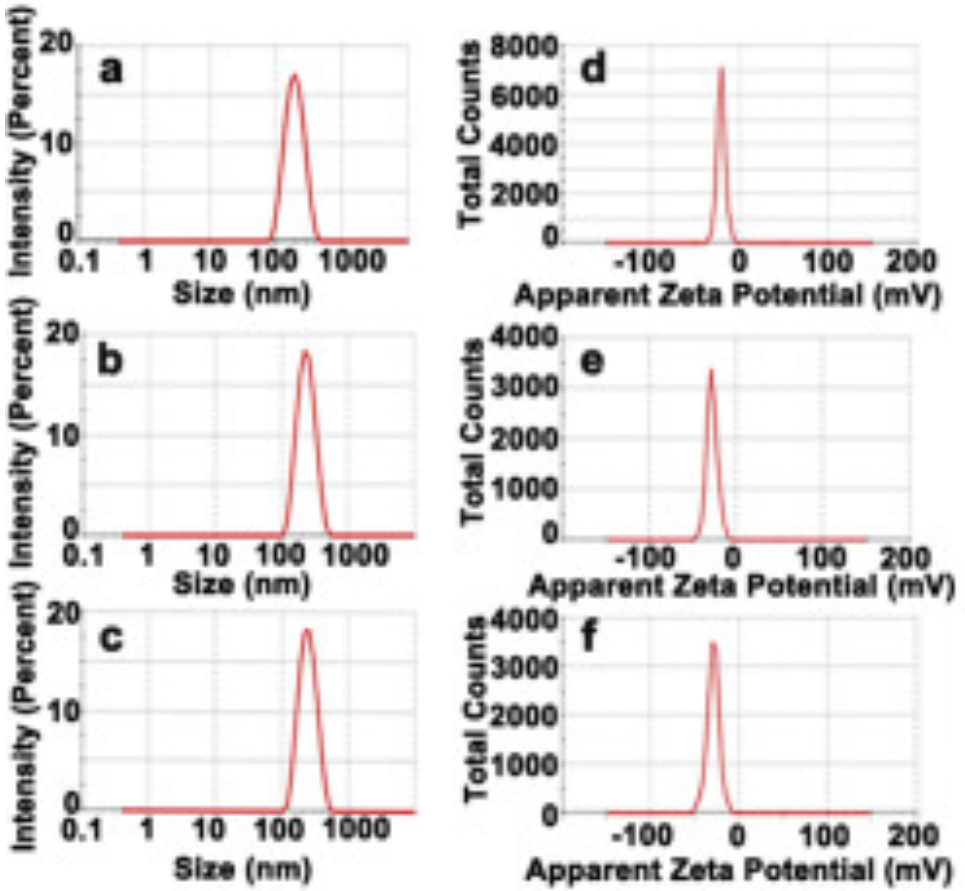
Hae-Yong Seok *et al. J. Controlled Release*, **2018**, *280*, 20–30.

Characterization of NPs (P14–P19)

TEM image



Size distribution & Zeta potential



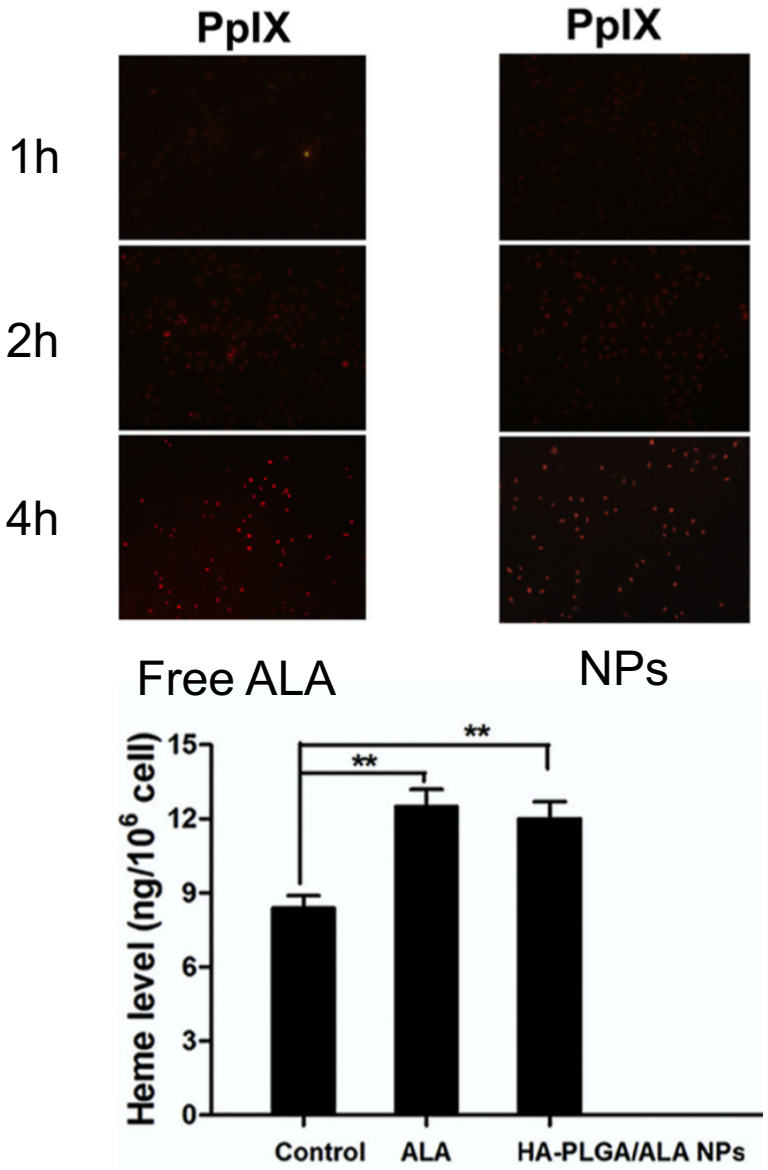
HA-PLGA

HA-PLGA/ALA

HA-PLGA @ART/ALA

Lei Wang, *et al. J. Controlled Release*, **2018**, 286, 74–84.

Cellular uptake and heme level (P14–19)



ALA is a precursor of PpIX, which shows red-fluorescence.

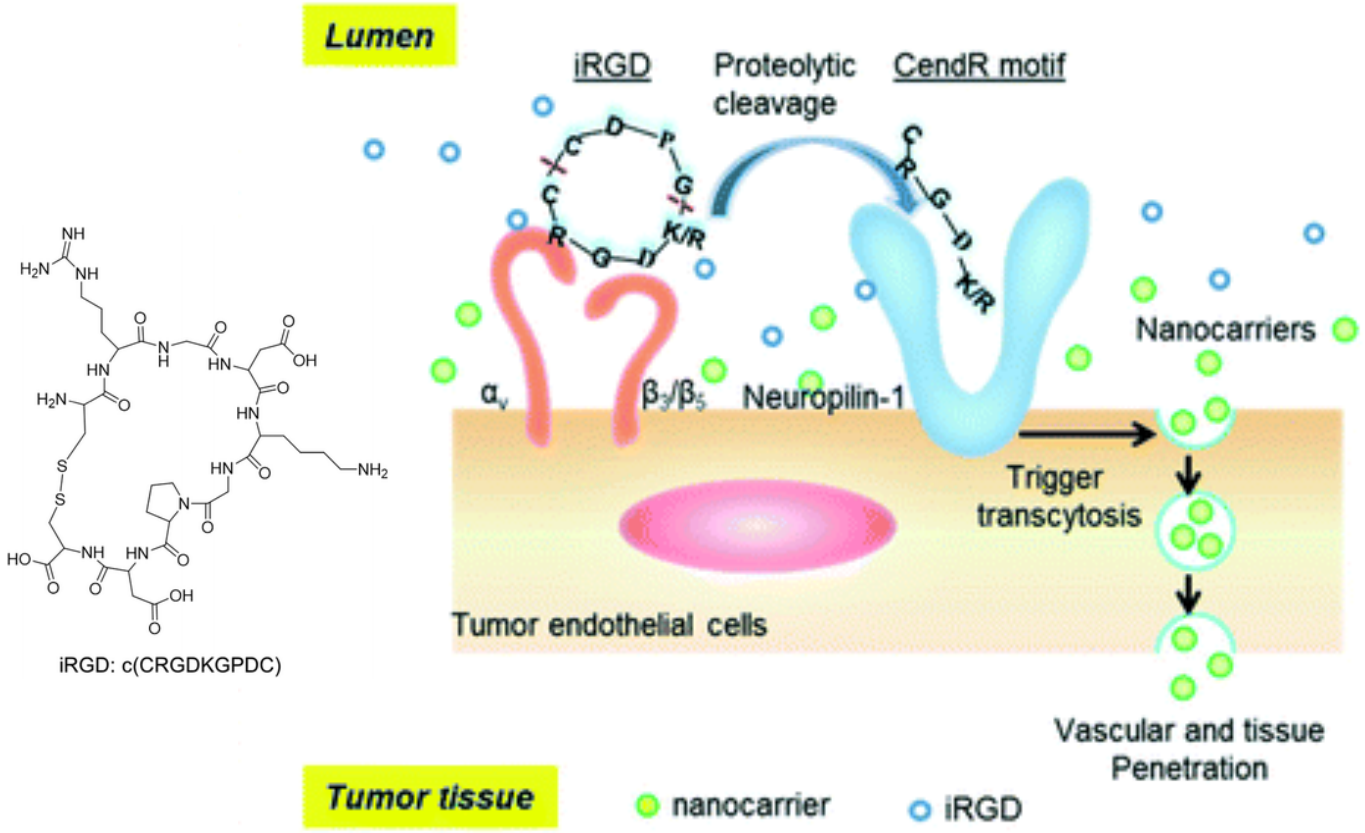
There was no remarkable difference between ALA and NPs.

→NPs could enter cells efficiently.

Both ALA and NPs increased heme level.

Lei Wang, *et al. J. Controlled Release*, **2018**, 286, 74–84.

Tumor targeting by iRGD (P22)

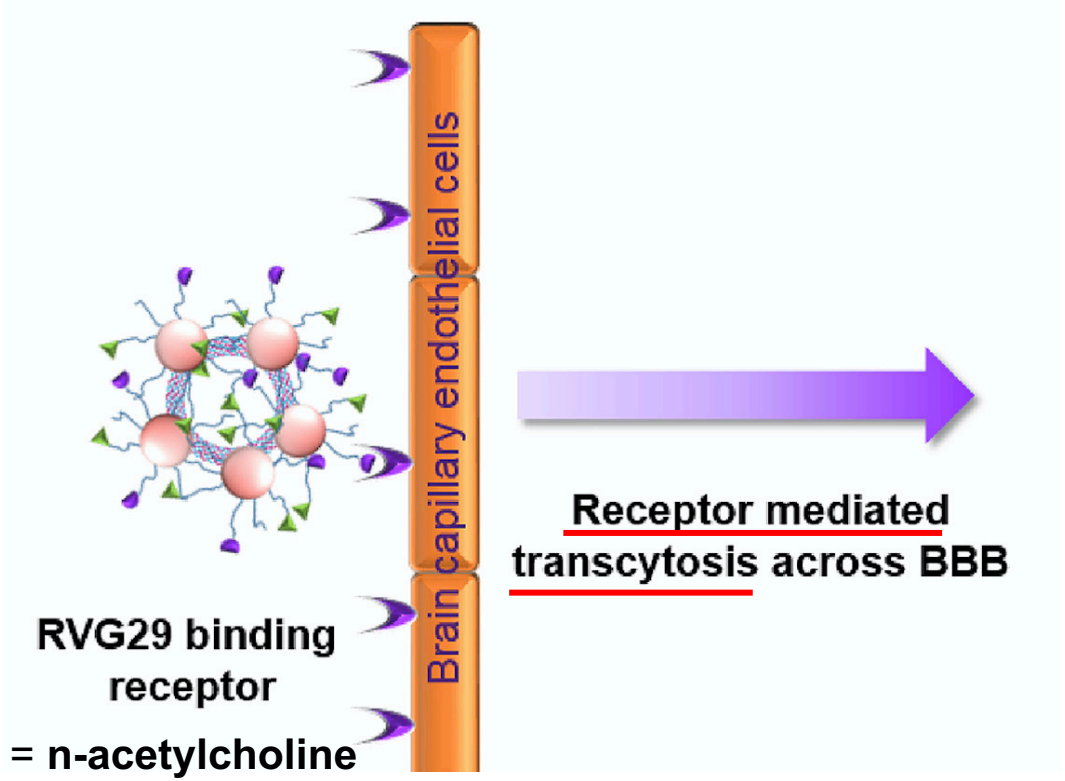


1. iRGD sequence binds to $\alpha v\beta_{3/5}$ integrin, which are specifically expressed in tumor vessels or tumor cells.
2. iRGD is hydrolyzed by host protease to expose CendR motif.
3. CendR interacts with neuropilin to initiate internalization.

Yue Sun *et al*. *Biomater. Sci.,* **2019**, *7*, 985–994.
Xiangsheng Liu *et al. Mol. Syst. Des. Eng*., **2017**, *2*, 370–379.

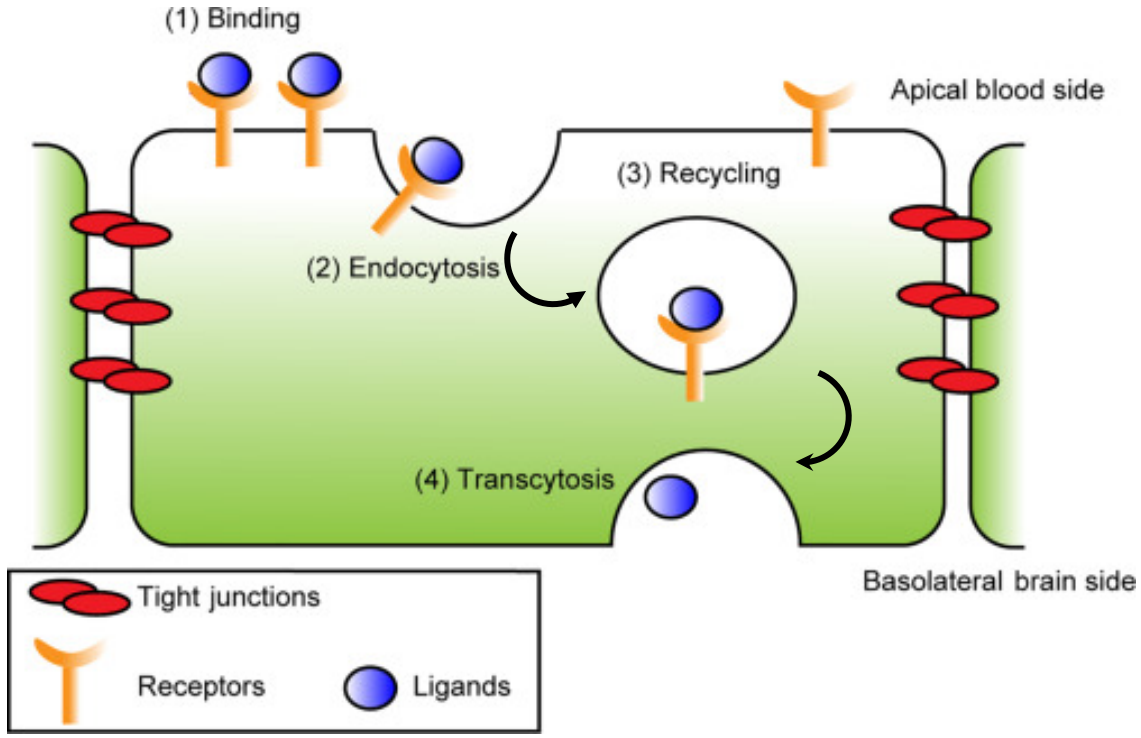
Rabies Virus Glycoprotein (RVG) drug delivery (P27)

The scheme of drug delivery by RVG peptide



Yang Liu *et al. Biomaterials*, **2016**, *80*, 33–45.

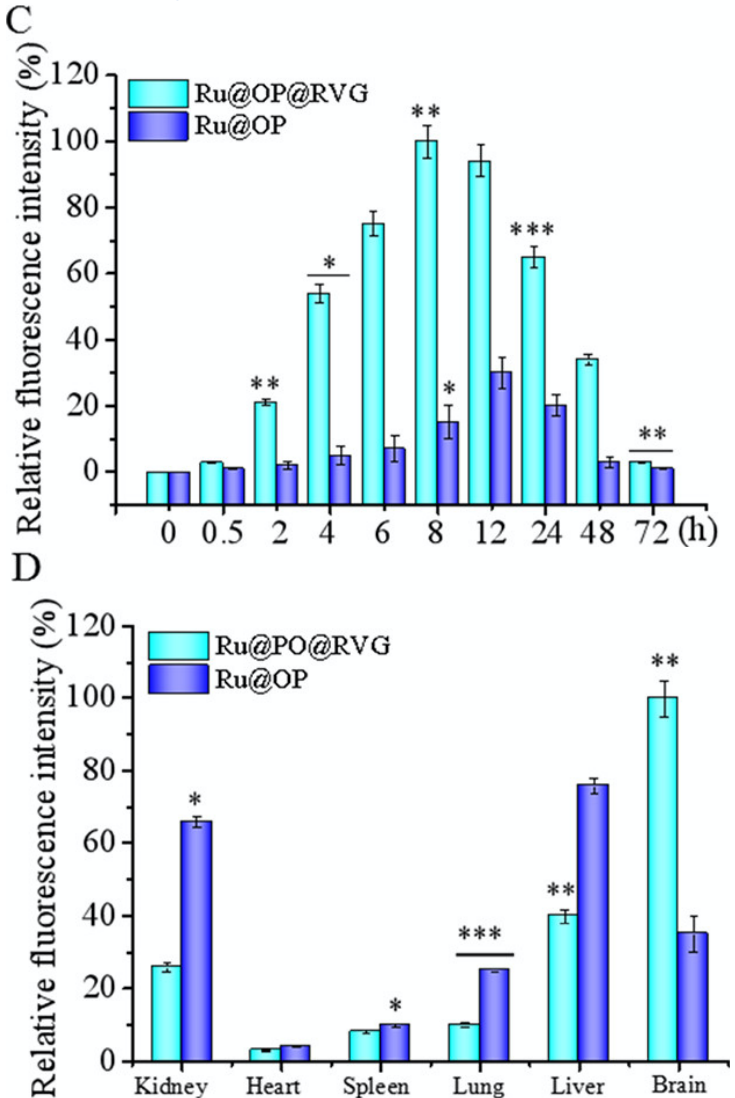
Receptor mediated transcytosis



Zhui Zhang, Changyou Zhan,
Brain Targeted Drug Delivery system,
2019, 105–128.

NP biodistribution (P26–30)

Quantitative analysis for the fluorescence *in vivo*



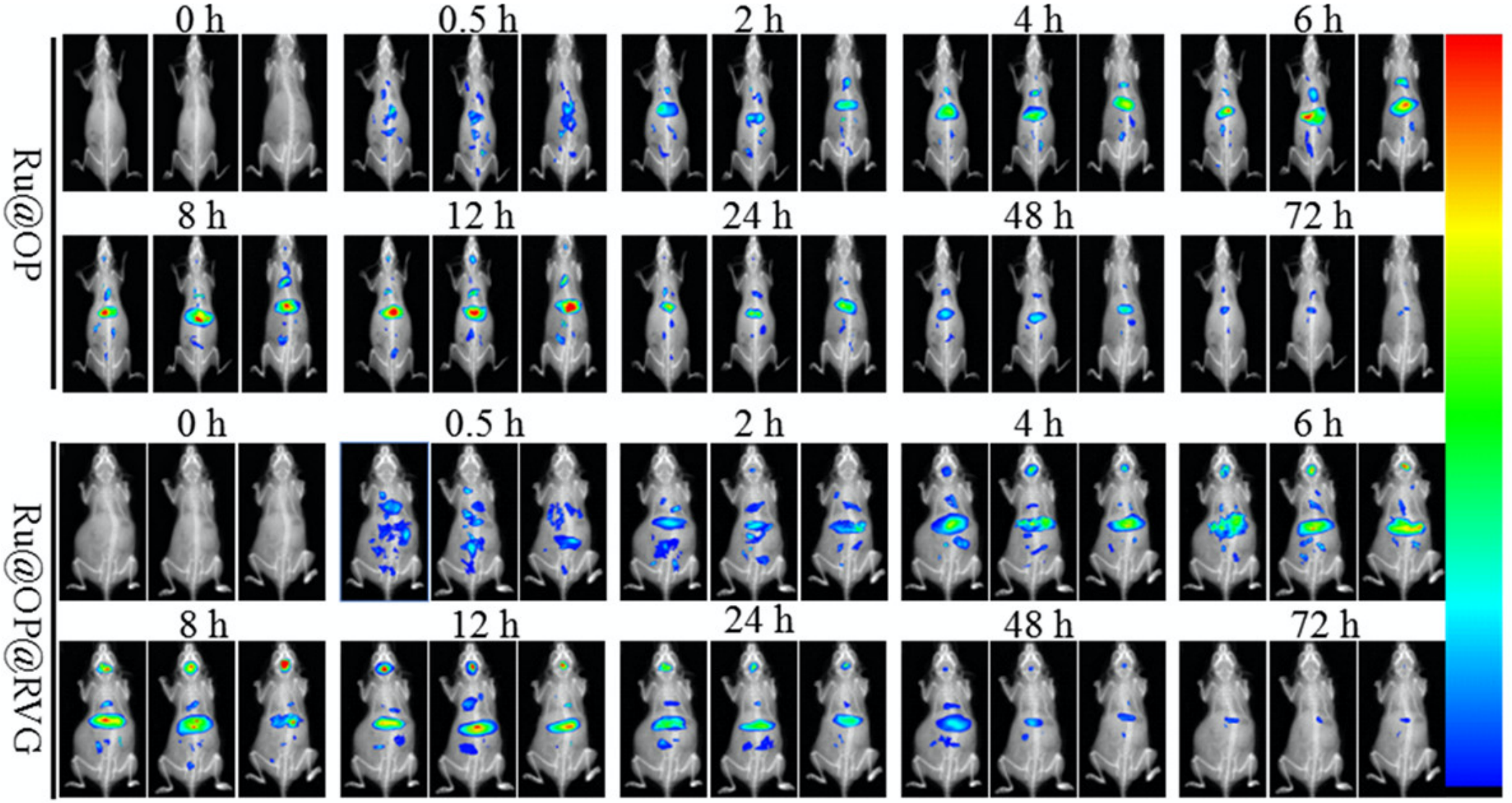
RVG-modified NPs had better circulation and retention efficiency.

NPs remarkably accumulated in the brain.

→RVG active targeting and improved penetration of BBB

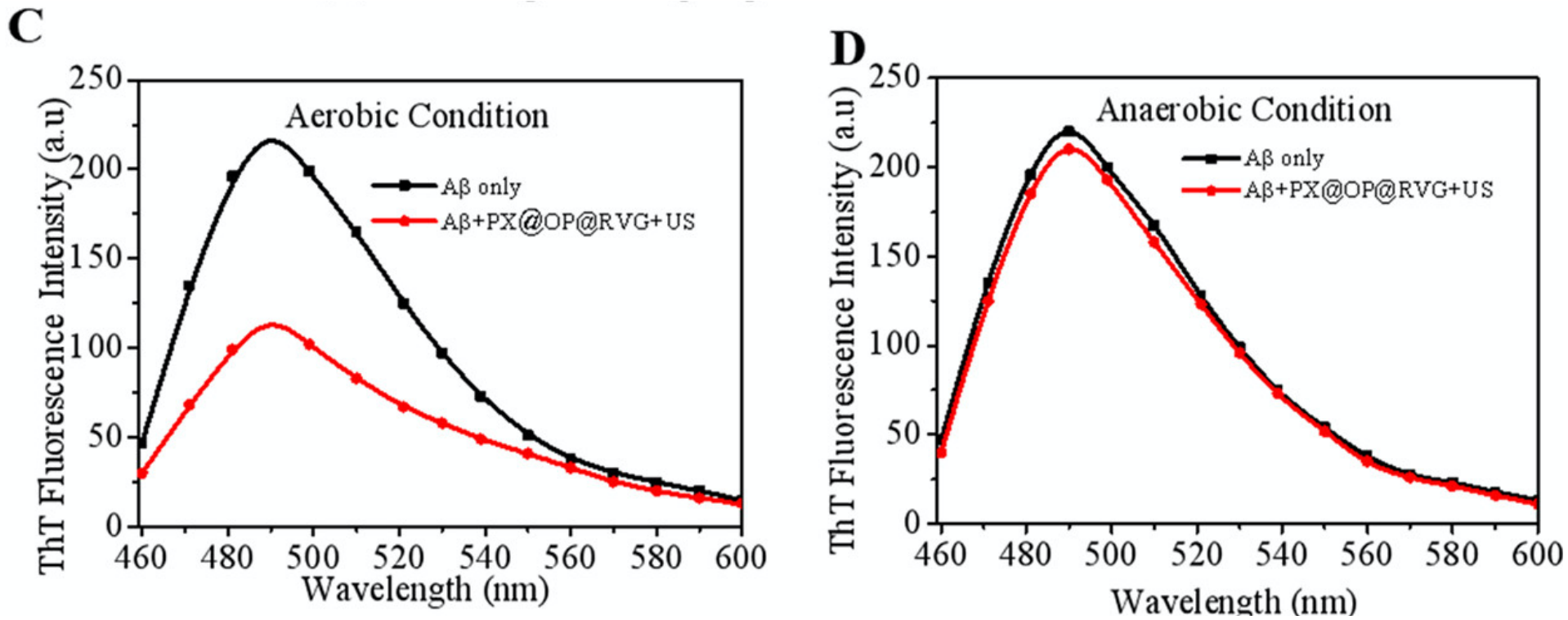
Mengmeng Xu *et al*. *ACS Appl. Mater. Interfaces*, **2018**, *10*, 32965–32980.

NP biodistribution (P26–30)



Mengmeng Xu *et al*. *ACS Appl. Mater. Interfaces*, **2018**, *10*, 32965–32980.

Inhibition of Aβ aggregation (P28)

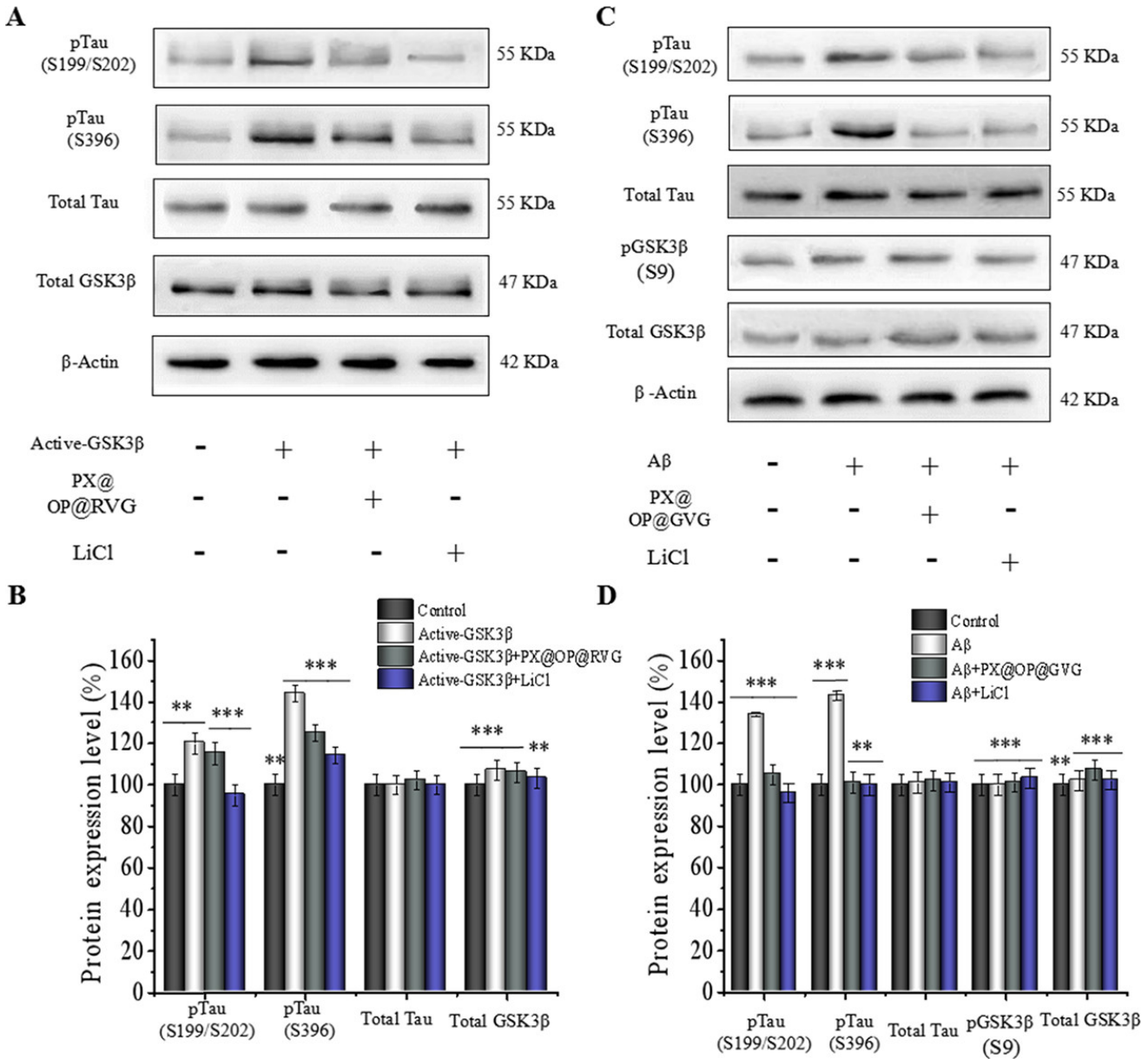


Anaerobic condition → None ROS production

ROS generation contributes to the inhibitory effect of US excited PX@OP@RVGs on Aβ aggregation.

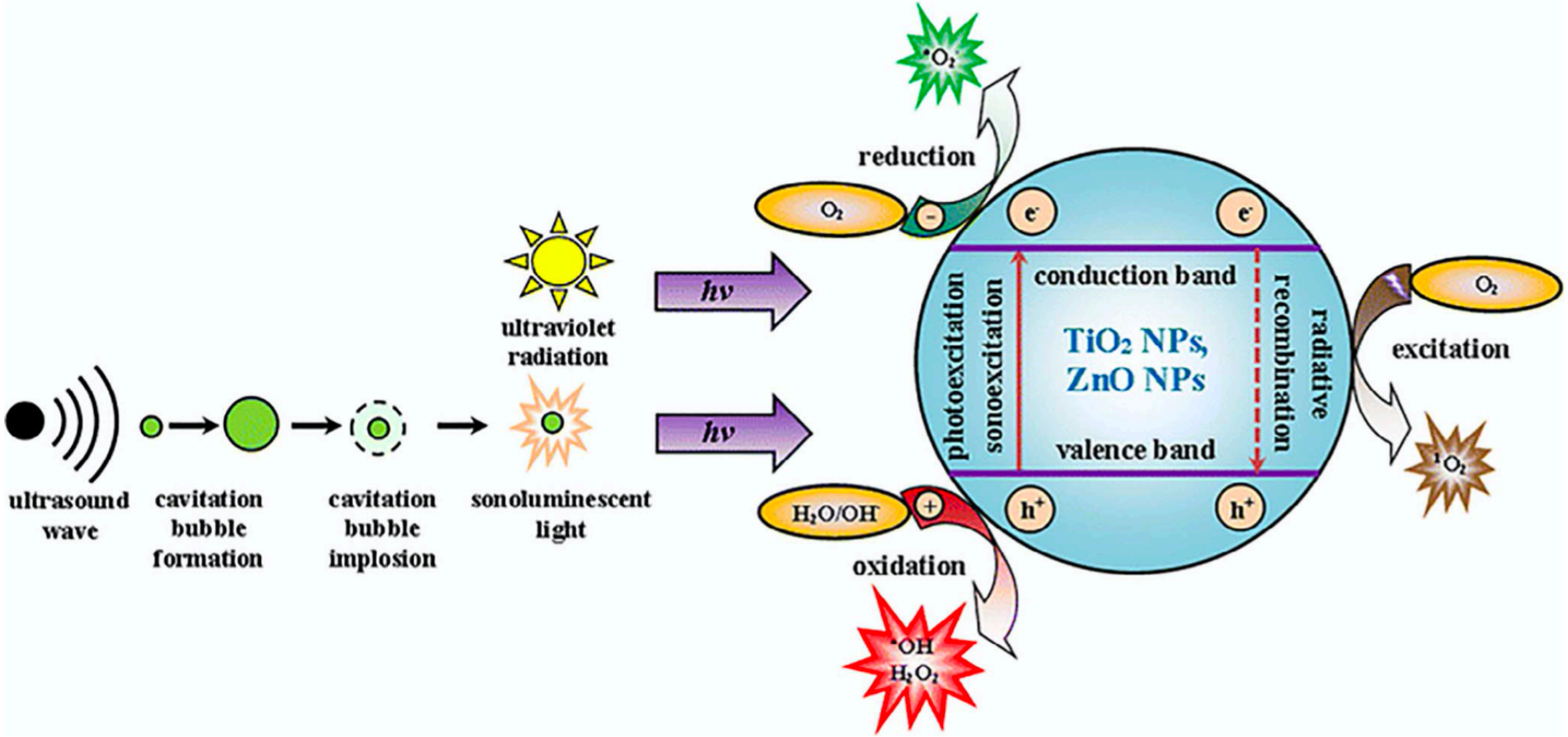
Mengmeng Xu *et al*. *ACS Appl. Mater. Interfaces*, **2018**, *10*, 32965–32980.

Inhibition of tau hyperphosphorylation (P29)



Mengmeng Xu *et al*.
ACS Appl. Mater. Interfaces, **2018**, *10*, 32965–32980.

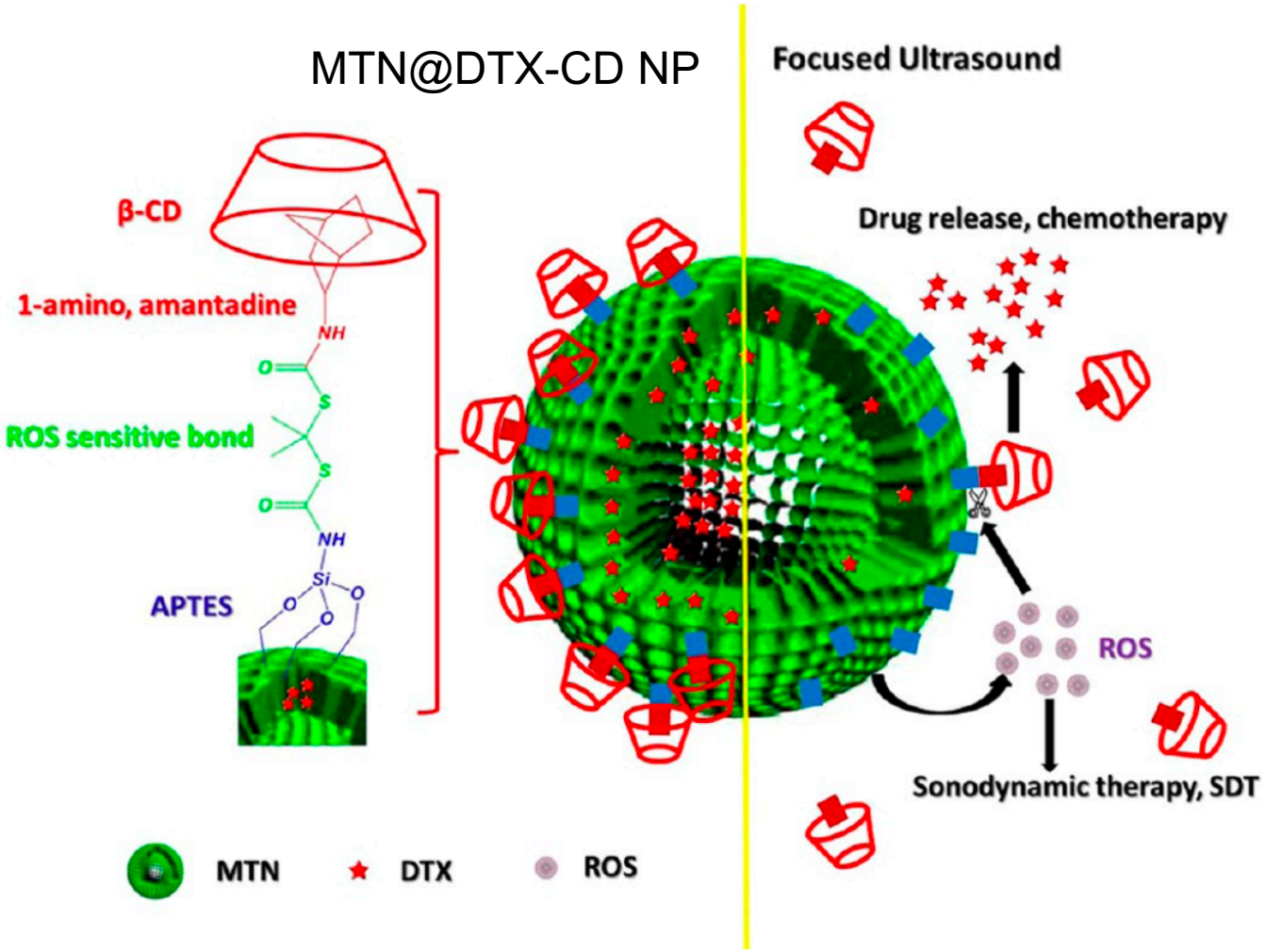
Inorganic sonosensitizer



Not only organic molecules but also inorganic NPs such as TiO_2, ZnO, etc. act as a sonosensitizer.

Chemical Engineering Journal, **2018**, *340*, 155–172

Mesoporous titanium nanoparticle loading DTX

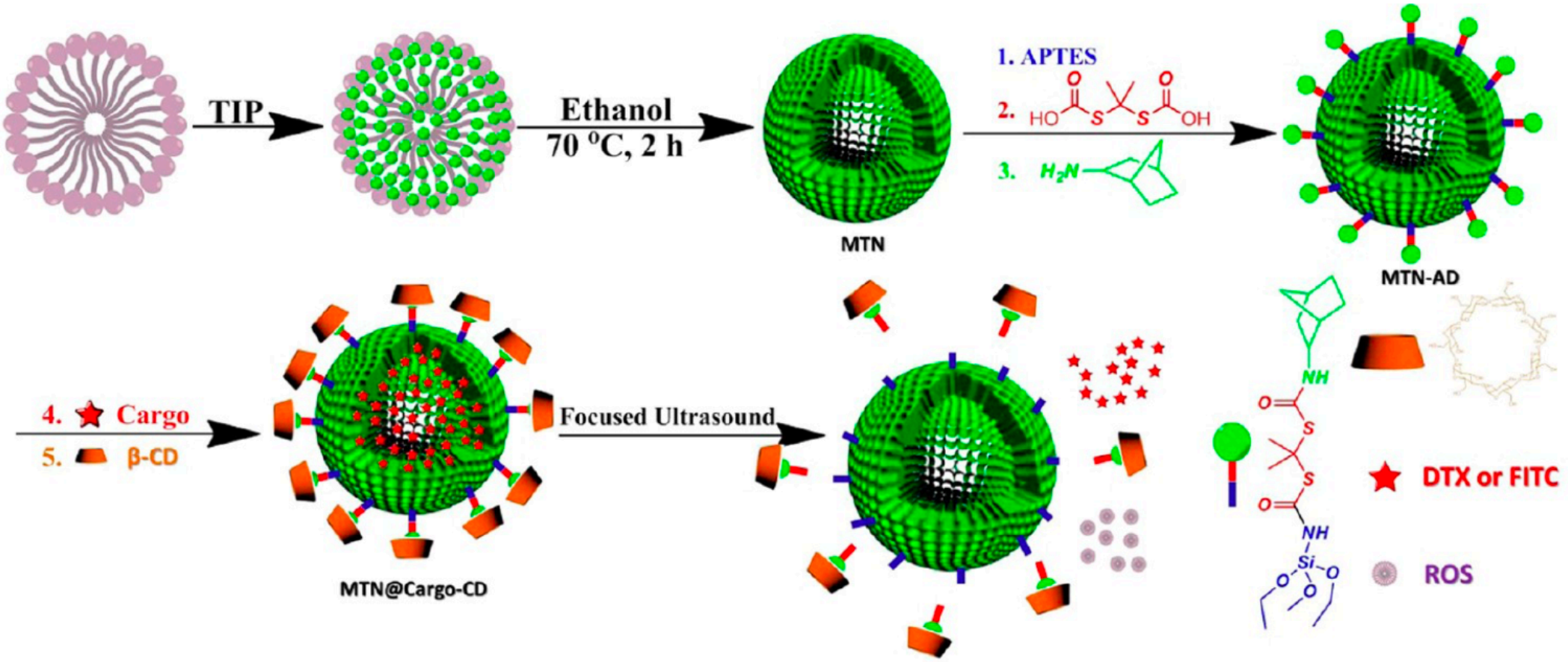


- Mesoporous TiO2 NPs (**MTN**): Carrier and sonosensitizer
- Docetaxel (**DTX**): Anti-cancer drug, loaded in pores
- *β*-cyclodextrin (***β*-CD**): A bulky gatekeeper to block mesopores

US-triggered drug release by ROS sensitive linker

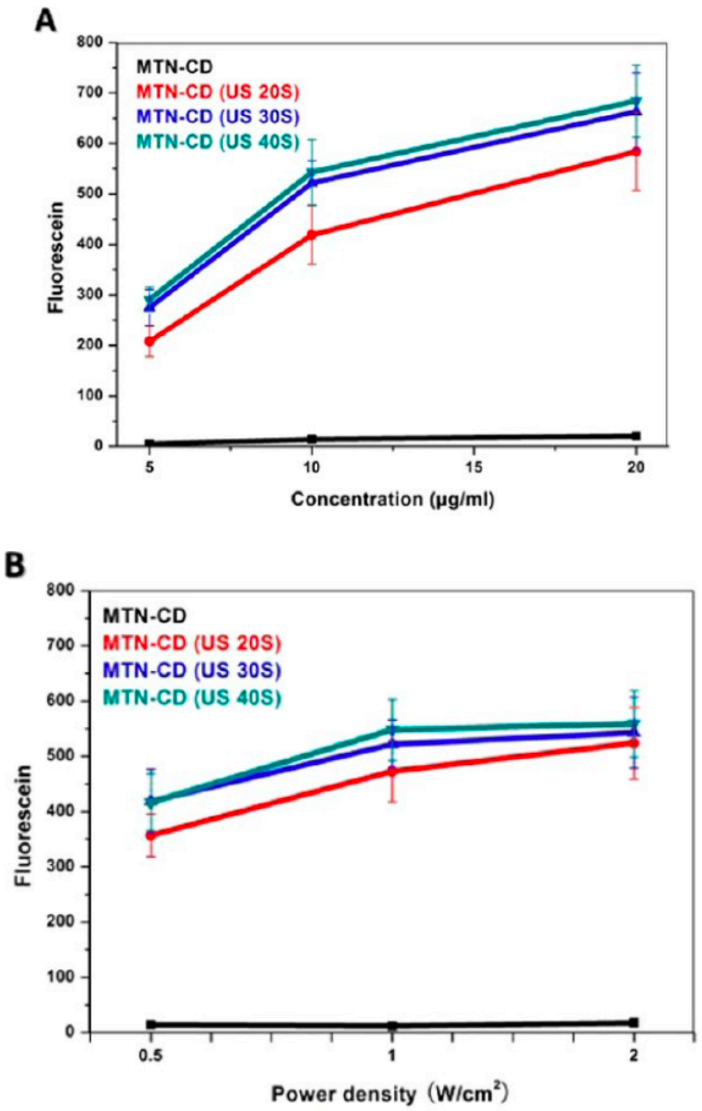
Jinjin Shi *et al. ACS Appl. Mater. Interfaces*, **2015**, *7*, 28554–28565.

Synthesis of MTN@DTX-CD NPs



Jinjin Shi *et al. ACS Appl. Mater. Interfaces*, **2015**, *7*, 28554–28565.

$^1O_2$ generation ability



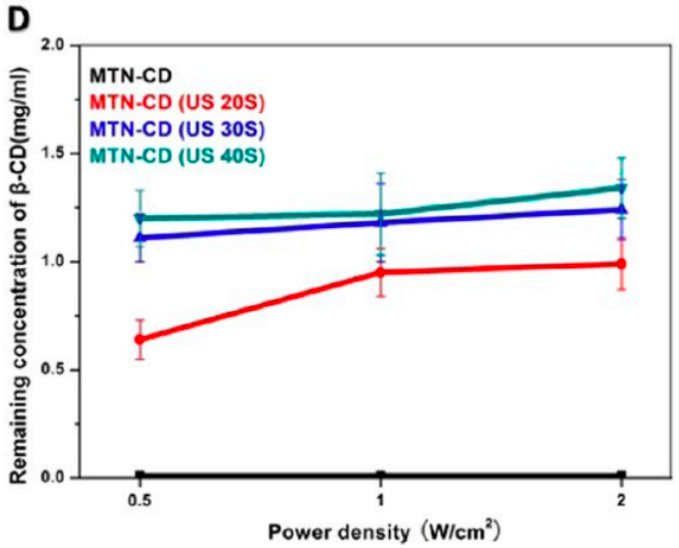
$^1O_2$ production was more efficient with increased concentration of MTN-CD.

US power density and irradiation time were also important.

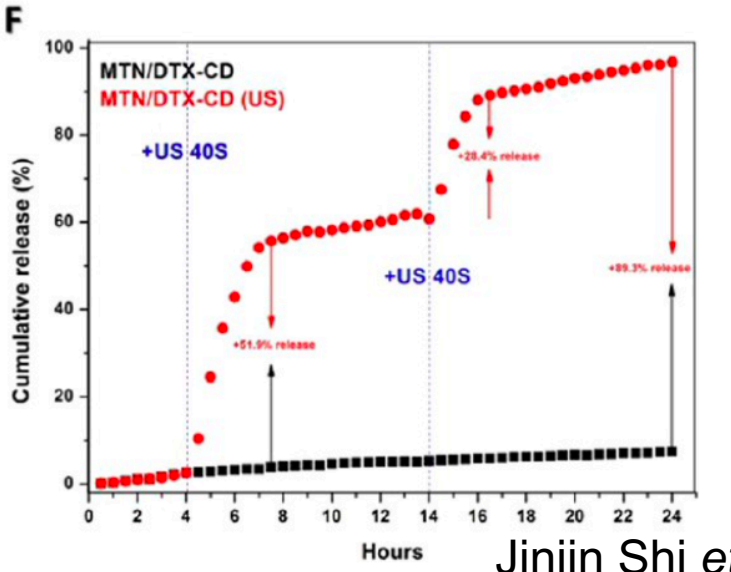
Jinjin Shi *et al. ACS Appl. Mater. Interfaces*, **2015**, *7*, 28554–28565.

US-triggered drug release

β-CD



DTX



β-CD disperse showed the cleavage of ROS-sensitive linker was occurred.

Rapid increase of DTX after US irradiation.

- 51.9% increase (4~7.5 h)
- 28.4% increase (14~17 h)

Jinjin Shi *et al. ACS Appl. Mater. Interfaces*, **2015**, *7*, 28554–28565.

Cell apoptosis assay

F Average percentages

Groups	Quadrant statistics (MCF-7 cells)		
	health	necrotic	apoptosis
Control	96.8±0.3	0.3±0.02	2.9±0.1
Control+US	92.2±2.1	4.5±1.1	3.3±0.3
MTN-CD	95.8±1.1	1.1±1.7	3.1±1.5
MTN-CD+US	47.3±3.9	8.1±1.4	44.6±4.1
DTX	68.9±2.1	1.8±0.3	29.3±0.9
MTN@DTX-CD	82.2±4.4	1.9±1.1	15.9±4.4
MTN@DTX-CD+US	29.7±4.9	6.8±3.2	53.5±4.4

← SDT only

← chemotherapy only

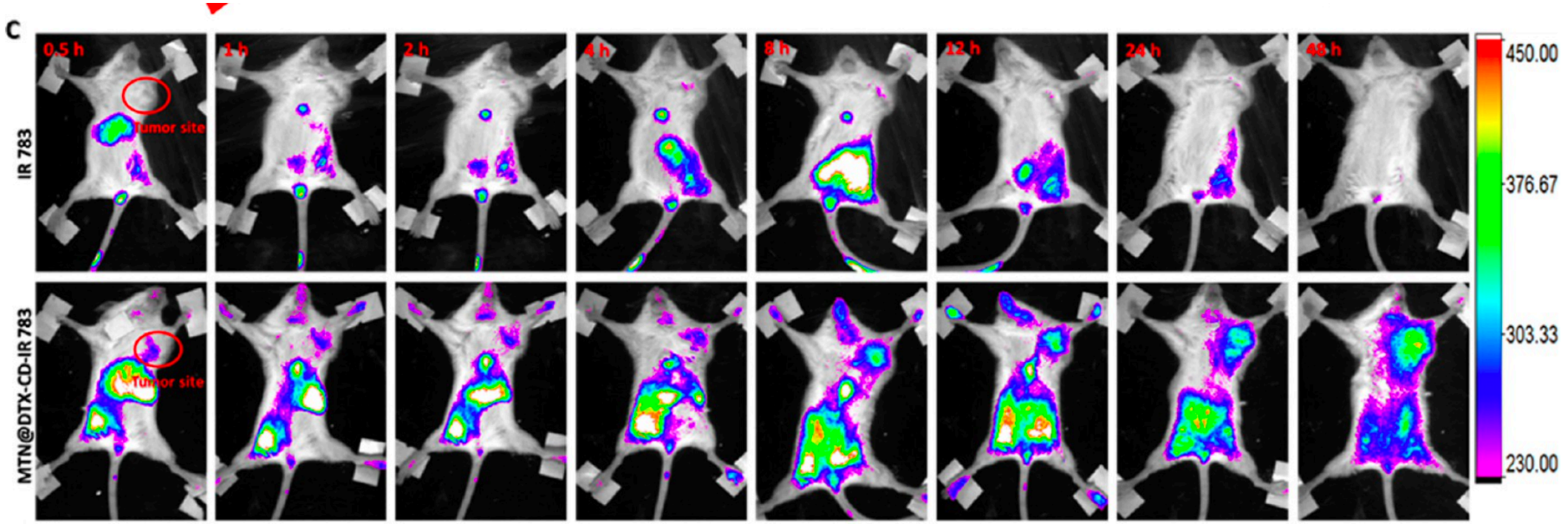
← SDT + chemotherapy

MTN@DTX-CD with US showed higher effect than the others.

→The combination of SDT & chemotherapy led to more apoptosis.

Jinjin Shi *et al. ACS Appl. Mater. Interfaces*, **2015**, *7*, 28554–28565.

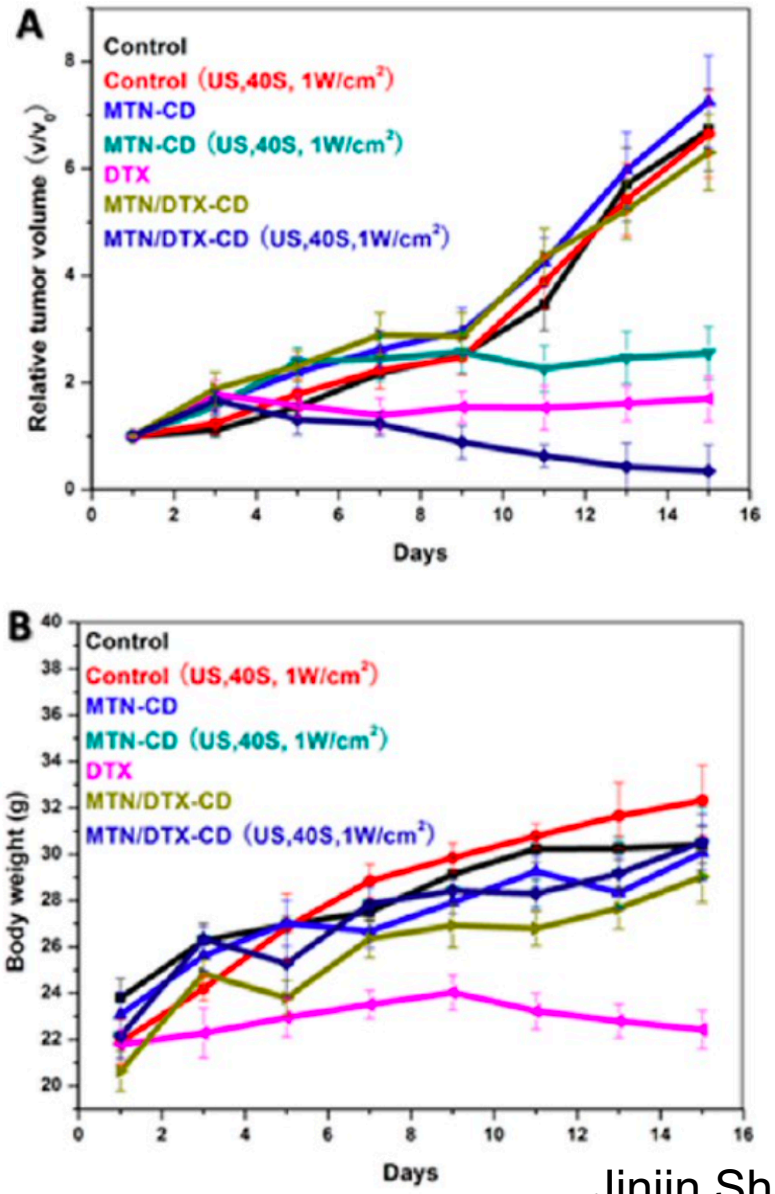
In vivo studies



- Tumor targeting and accumulation
- Long circulation ability

Jinjin Shi *et al. ACS Appl. Mater. Interfaces*, **2015**, *7*, 28554–28565.

In vivo studies



MTN-CD+US: SDT

DTX: chemotherapy

MTN/DTX-CD+US: the combination of SDT & chemotherapy

→More effective and less toxic side effect

Jinjin Shi *et al. ACS Appl. Mater. Interfaces*, **2015**, *7*, 28554–28565.