Conjugated polymer nanoparticles for theranostic applications

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Slides available here:

Outline

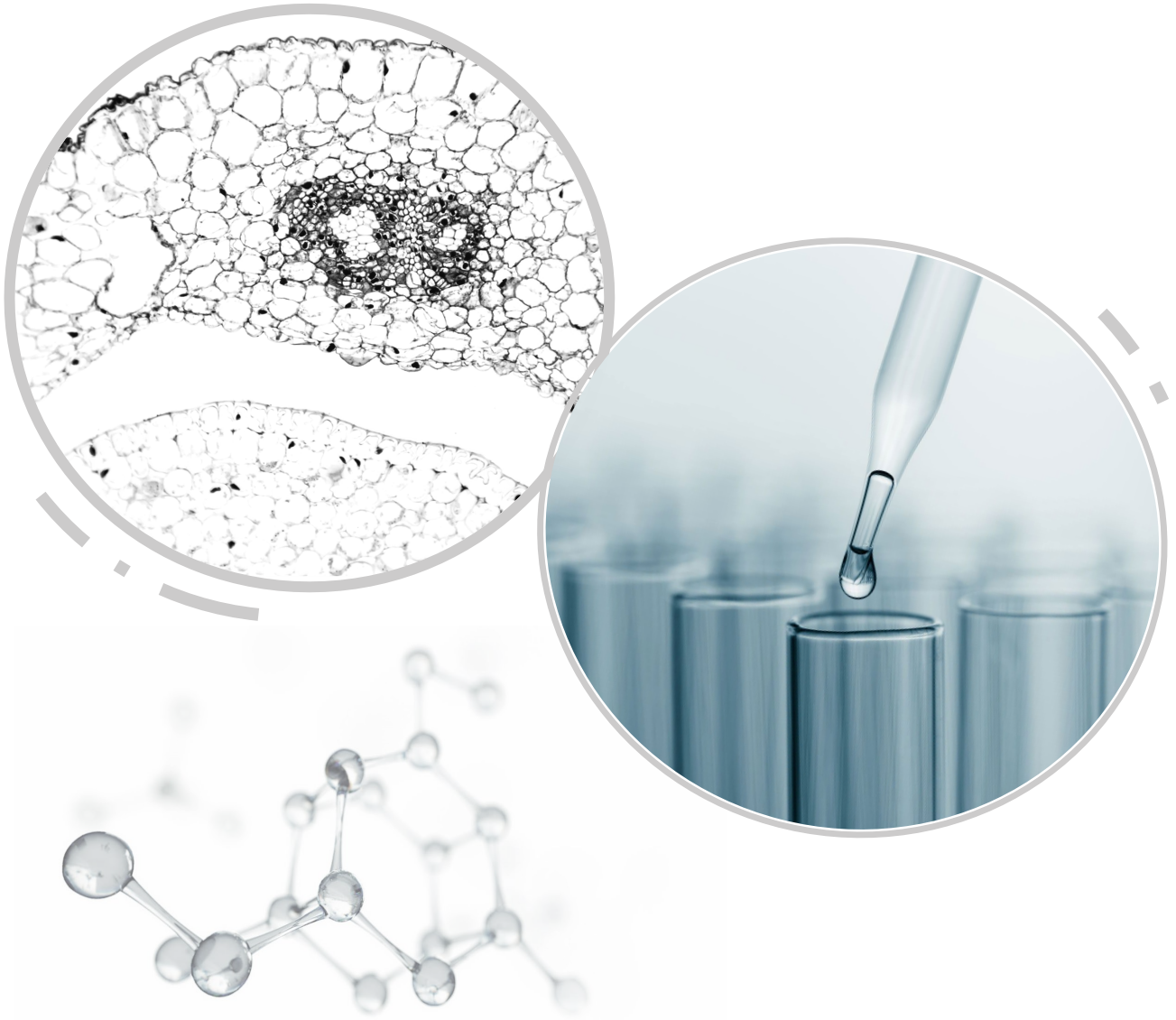
Introduction:

- Theranostics
- Theranostic probes
- Nanomaterials for theranostics

NIR-active theranostic probes:

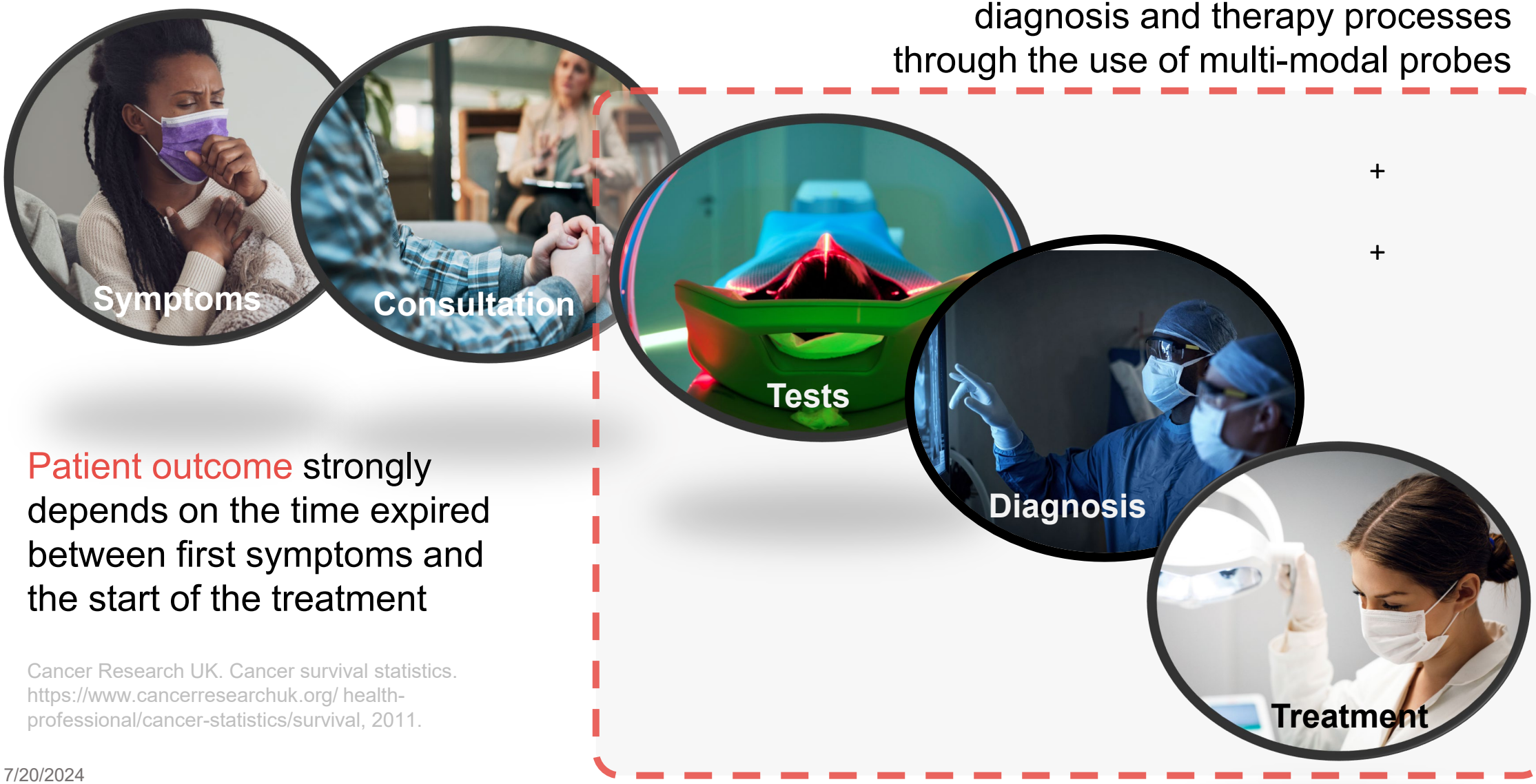
- CPN fabrication
- Physico-chemical properties
- Efficiency as a PDT agent
- Specificity

Conclusions & future outlook



Motivation

Theranostics aims to combine the diagnosis and therapy processes through the use of multi-modal probes



Patient outcome strongly depends on the time expired between first symptoms and the start of the treatment

Cancer Research UK. Cancer survival statistics. https://www.cancerresearchuk.org/ health-professional/cancer-statistics/survival, 2011.

Theranostics = diagnosis + therapy

Theranostic probes:

- ✓ Diagnostic modality — e.g. MRI, fluorescence, staining, acoustic, PET
- \+
- ✓ Therapeutic modality — e.g. chemotherapy, photodynamic therapy, thermal ablation

Other (ideal) probe properties:

- ✓ Long shelf-life
- ✓ Soluble in biological fluids
- ✓ Therapeutic modality activatable
- ✓ Chemically modifiable
- ✓ Size appropriate for body clearance
- ✓ Inherently not cytotoxic

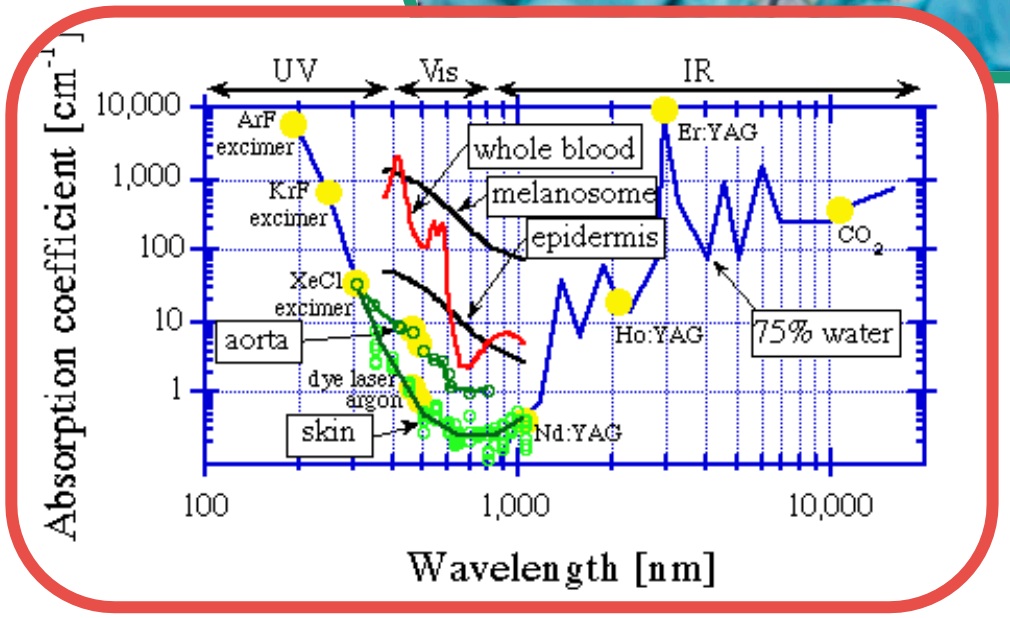
Optical excitation-based theranostic probes

Low-cost sources available ✓

Real-time imaging ✓

Compatible with endoscopy ✓

Image from https://mackinawsurgerycenter.com/colon-rectal/what-is-an-endoscopy-and-why-would-my-doctor-order-one/

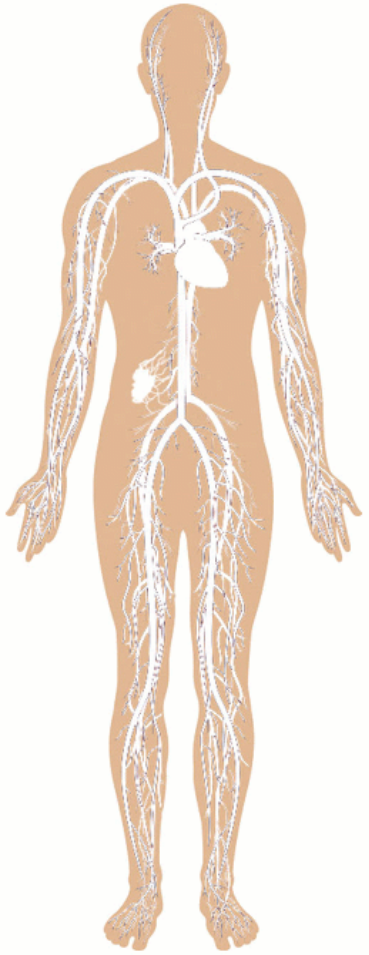


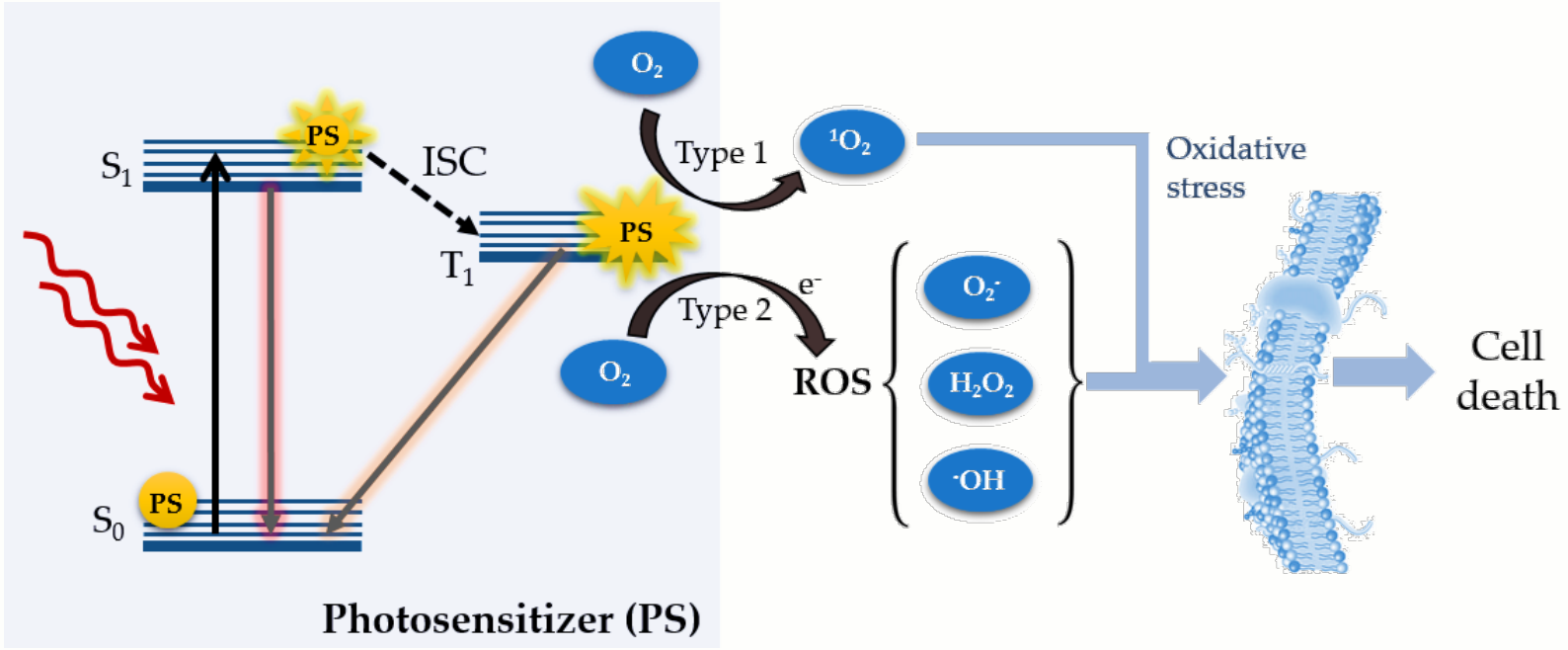
Graph from: ECE532 Biomedical Optics, © 1998 Steven L. Jacques, Scott A. Prahl, Oregon Graduate Institute

- ✘ Absorption by tissues
- ✘ Relies on visual interpretation
- ✘ Few phototherapy options

Photodynamic therapy

Relies on photo-initiated sensitization



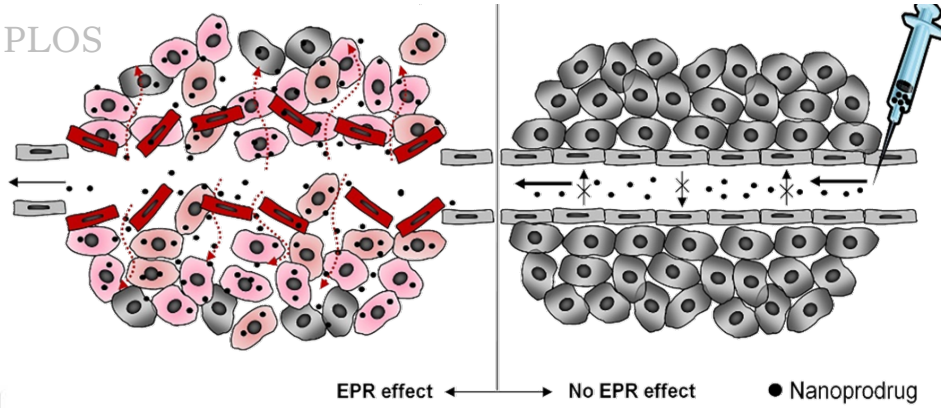


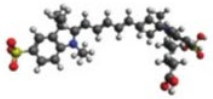
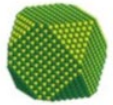
PDT agents in use today:

- Organic molecules
- Short shelf-life
- Shallow penetration depth
- Poor selectivity

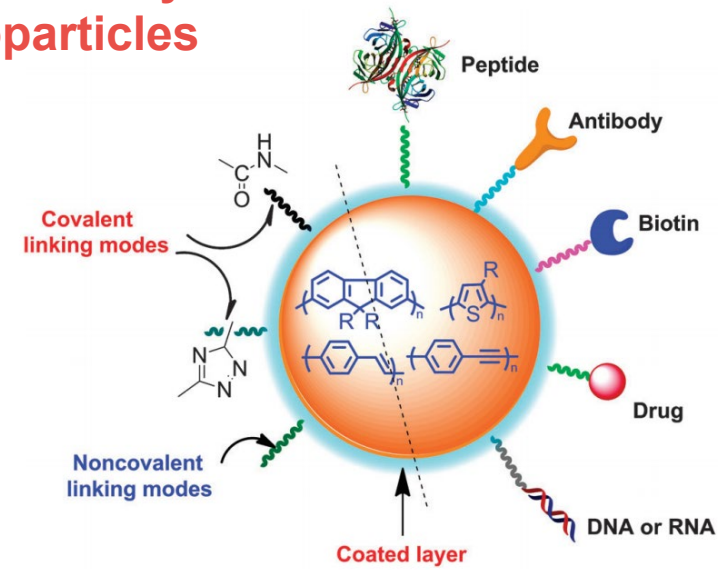
Nanomaterials as theranostic probes

- Some are fluorescent
- High extinctions
- Can be made stable/soluble in water
- Appropriate size
- Further surface modification often possible



	CLASS	SUB-CLASS	EXAMPLE / DESCRIPTION
	Molecules/ polymers	Organic fluorophores Fluorescent proteins Polymeric materials Fluorescent beads	Indocyanine green, Cy7 mNeptune, TagRFP657 Polyfluorene, poly(p-phenylenevinylene) Dye doped polymer, lipid or silica NPs
	Semi-conductor	Core type QDs Core/shell QDs Alloyed or doped QDs Silicon QDs	PbS, CdTe CdSe (core) / ZnS (shell) $CuIn_xSe_Y$, Cu doped InP Si nanoparticles
	Carbon materials	Nanodiamonds Carbon dots Graphene related	NV and Si color center in diamond Graphite particle with high O and N content Graphene oxide, SWCNTs
	Metal clusters	Gold clusters Silver clusters Copper clusters	Few tens of metal atoms, stabilized in solution by ligands
	Rare-earth NPs	Upconverting NPs Downconverting NPs	Er^{3+} or Yb^{3+} doped $NaYF_4$ Ho^{3+} doped $NaYF_4$

Conjugated Polymer Nanoparticles

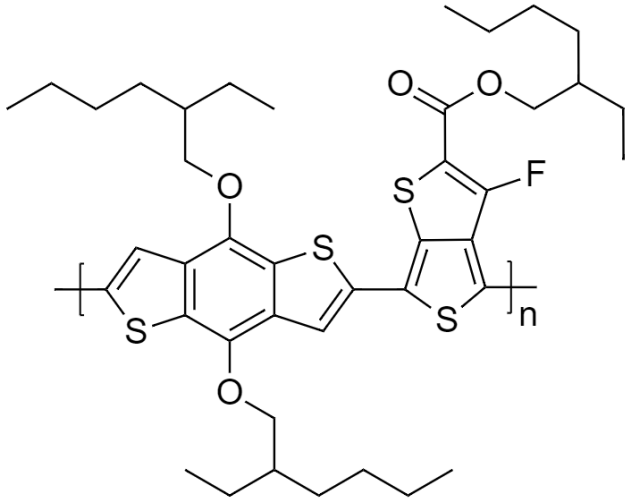


Advanced Optical Materials, 5, 1600446 (2017).
Chemical Society Reviews, 42(41), 6620-6633.

Fabrication of CPN-based probes

Probe constituents

Conjugated Polymer
(hydrophobic)

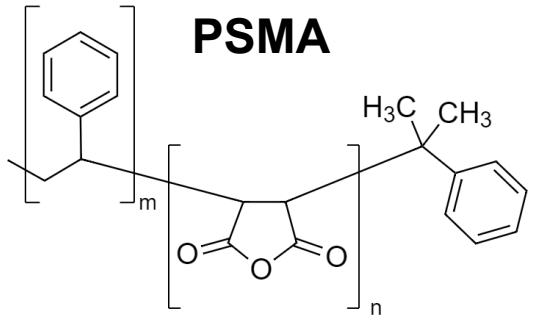


PTB7
known singlet oxygen producer

+

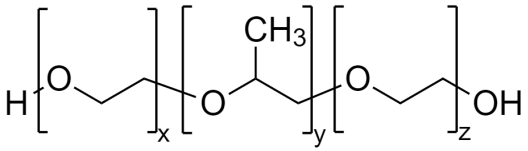
Copolymer
(amphiphilic)

PSMA

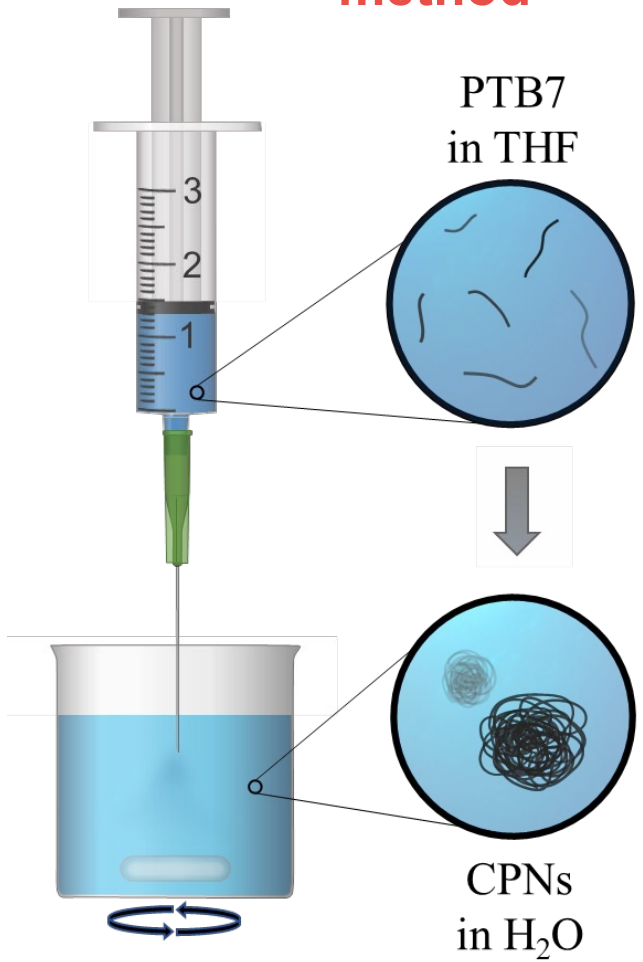


or

F127



Nanoprecipitation method



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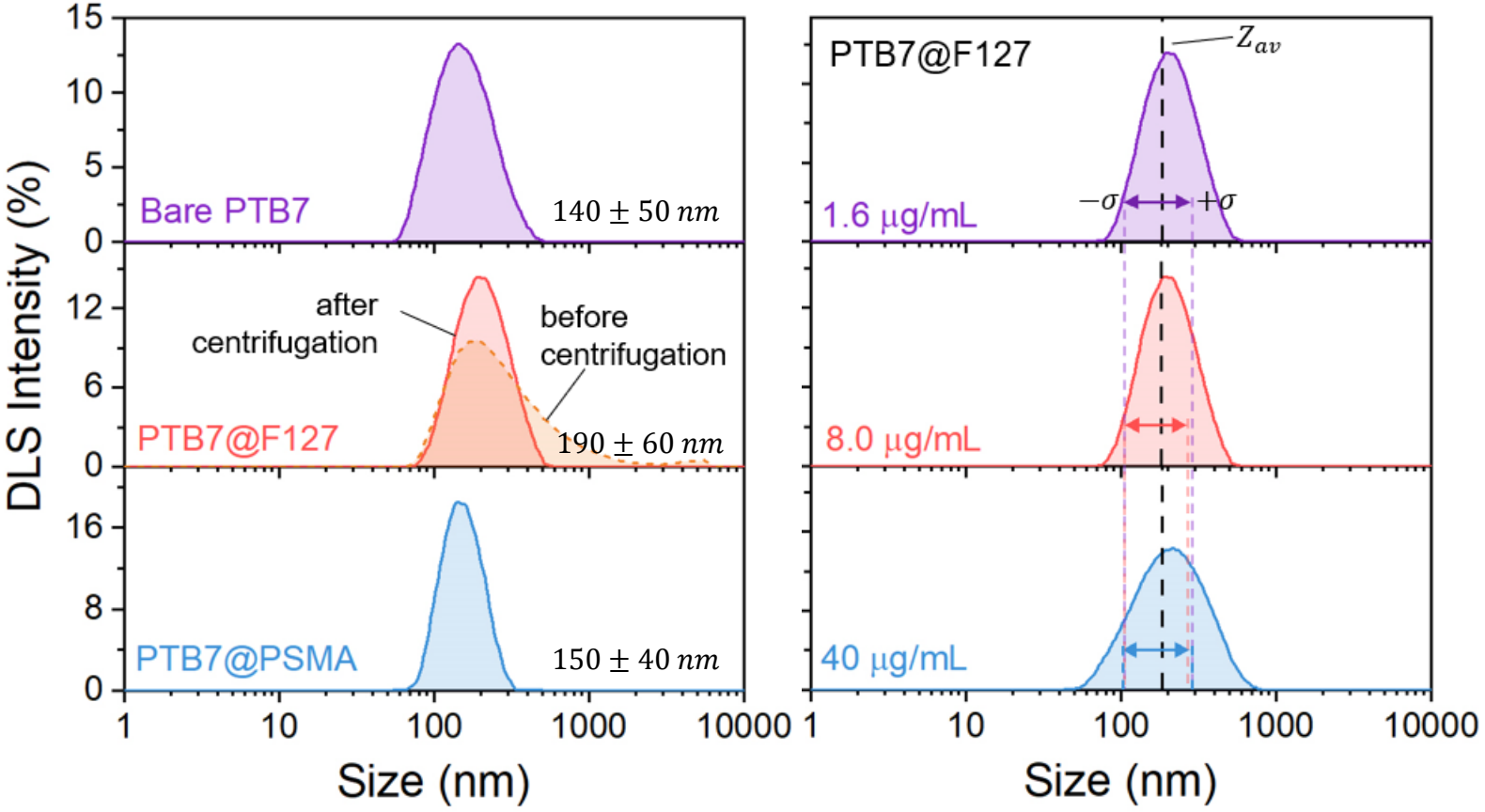
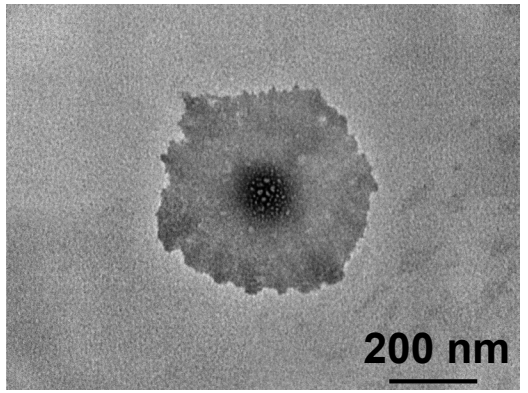
Size of CPN-based probes

Dynamic Light Scattering

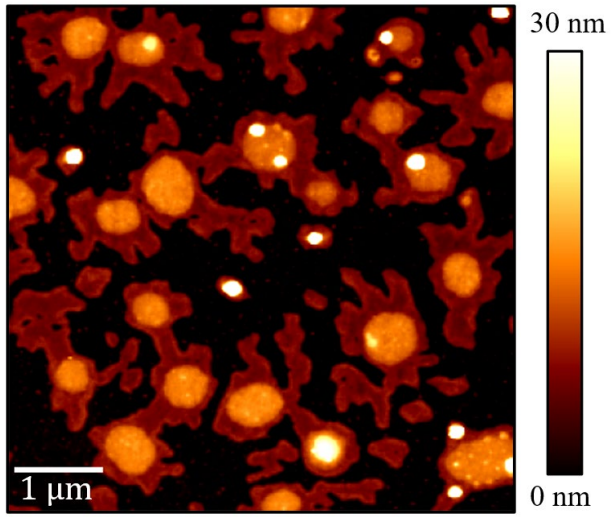


DLS size within the target window for body clearance

SEM



AFM



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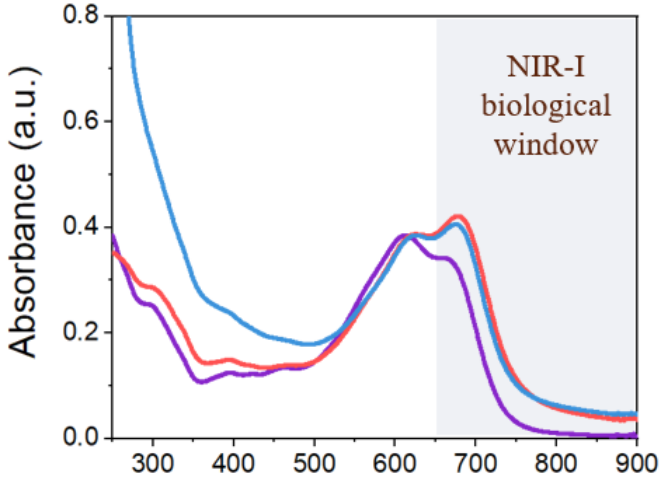
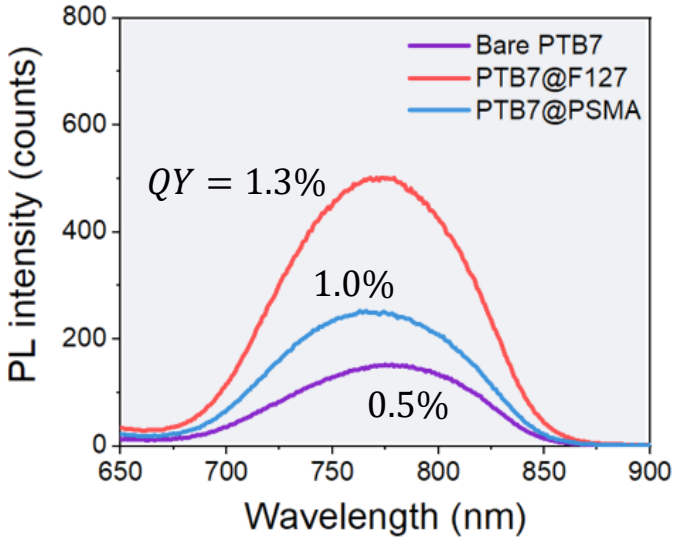
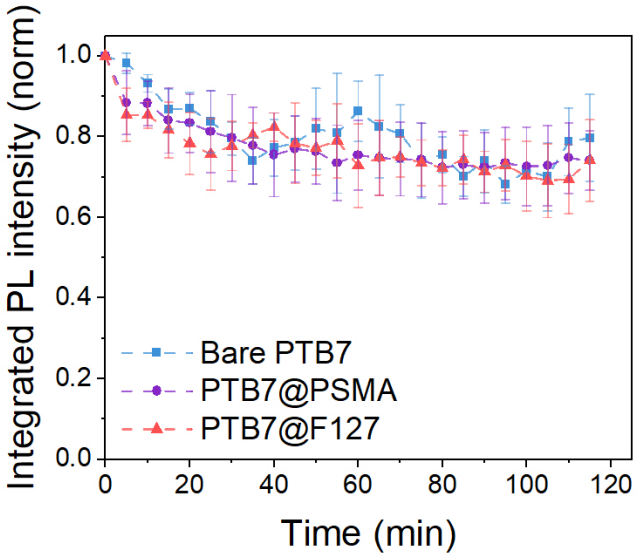
Optical properties vs bioimaging requirements

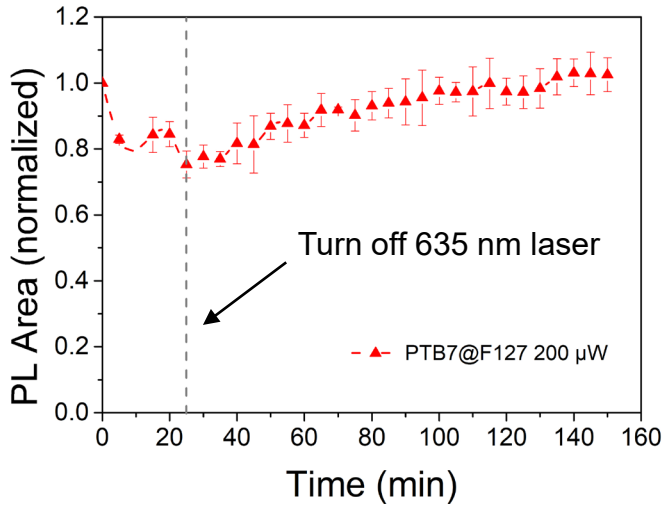
Avoiding tissue absorption



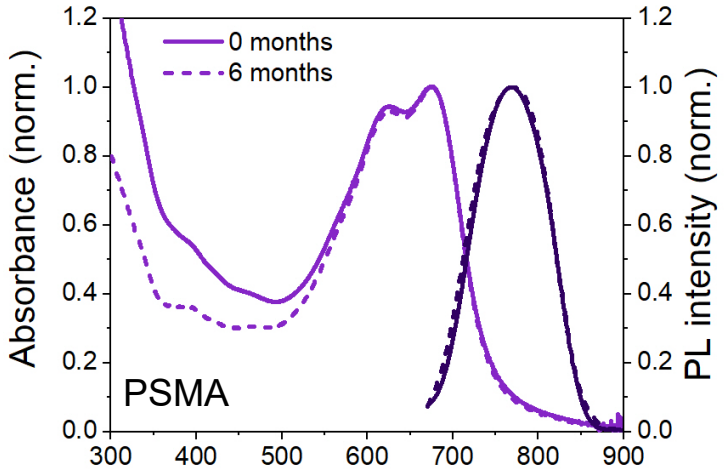


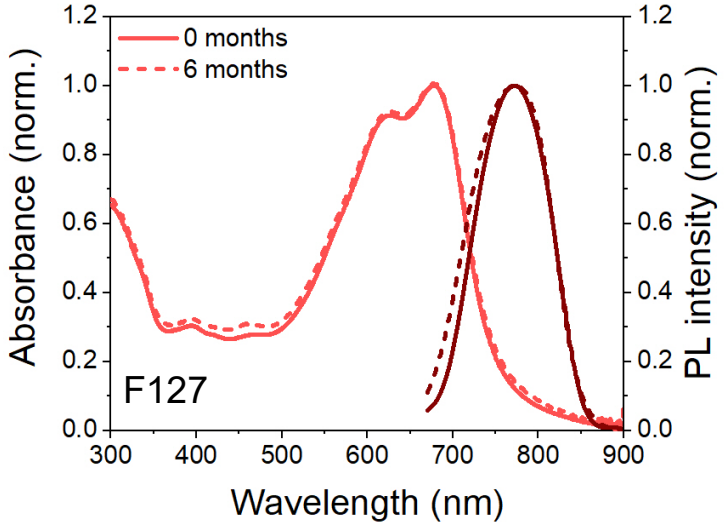
Resistance to photobleaching





Long-term photostability





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Measurements of photodynamic efficacy *ex vitro*

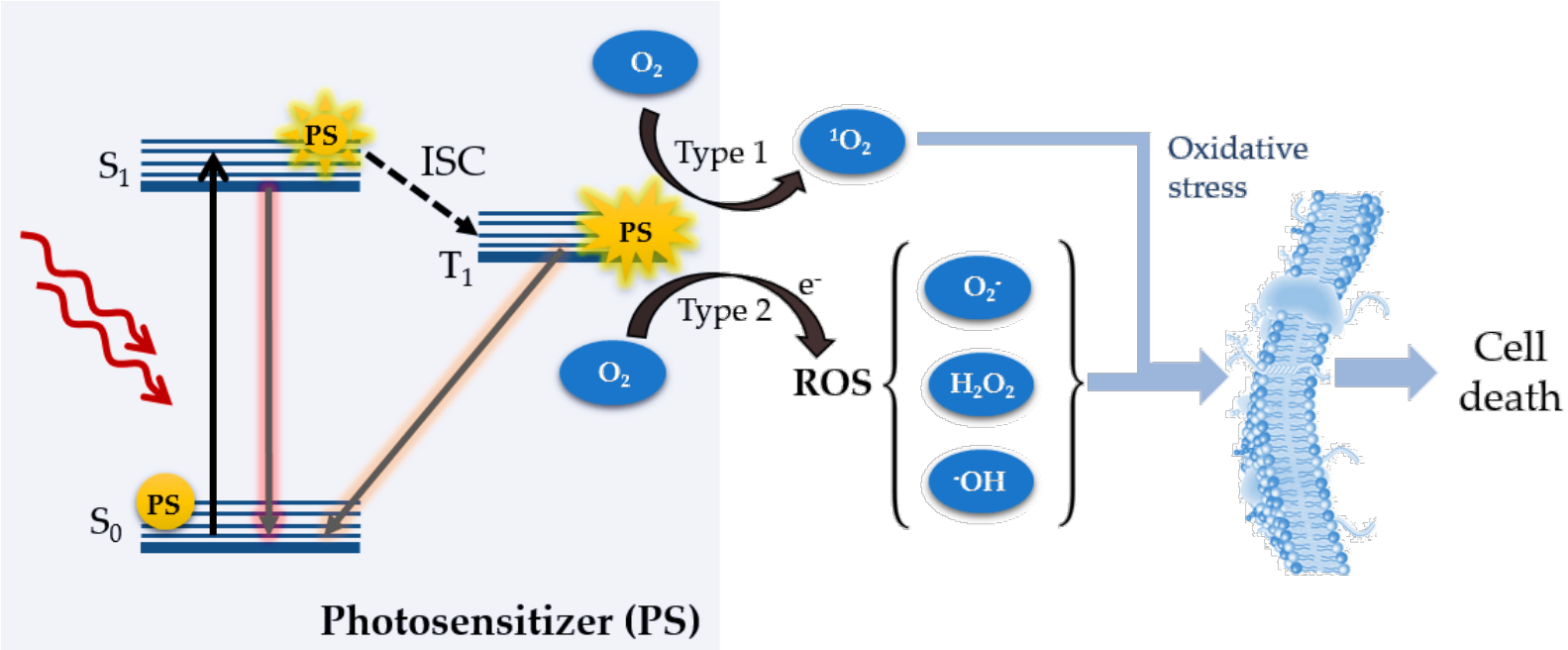
Types of Reactive Oxygen Species

Test for using an electrochemical detection method →

$^1O_2$	**Singlet oxygen**
HO•	Hydroxyl radical
$•O_2^-$	Superoxide anion
$ONOO^-$	Peroxynitrite anion
ROO•	Peroxyl radical
NO	Nitric oxide
HOCl	Hypochlorous acid
H_2O_2	Hydrogen peroxide

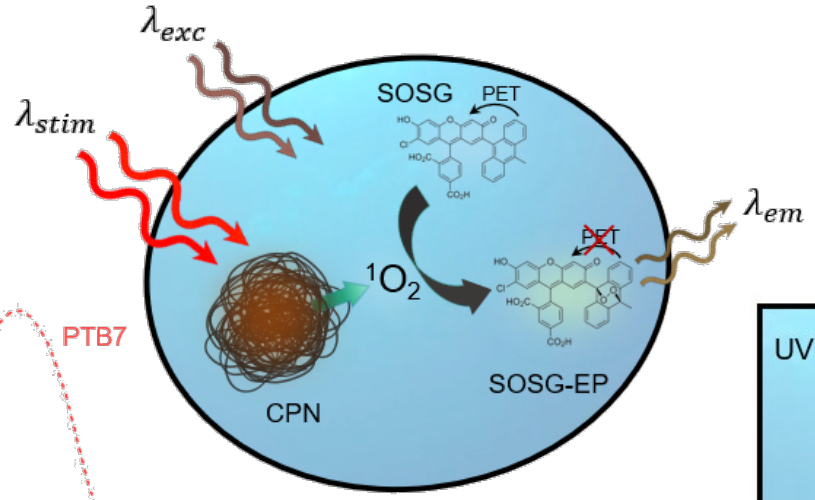
← Test for using a relative spectroscopic method

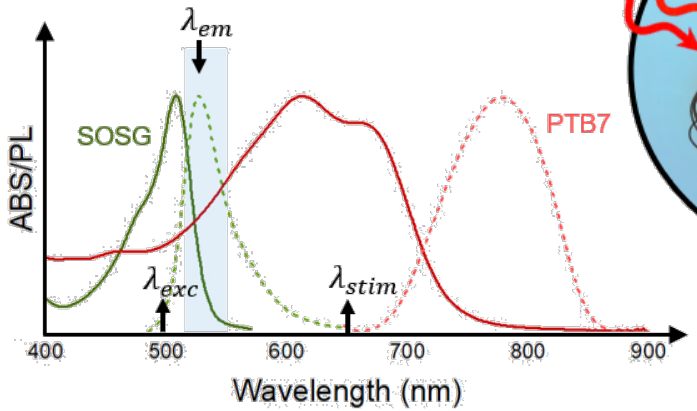
- $^1O_2$ is the major cytotoxic agent in photodynamic therapy.

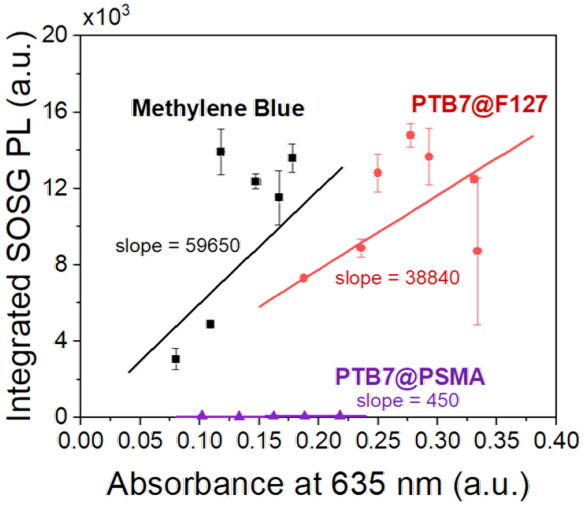


ROS production by PTB7 CPNs *ex vitro*

Singlet oxygen



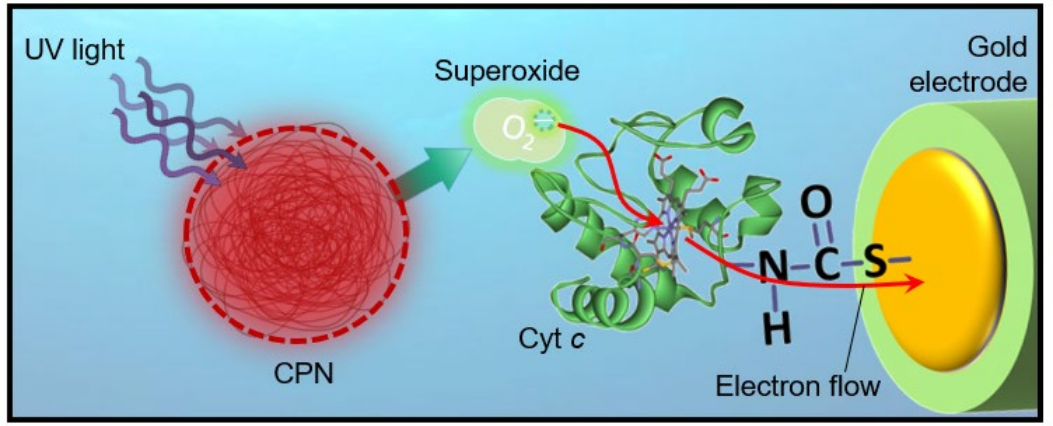


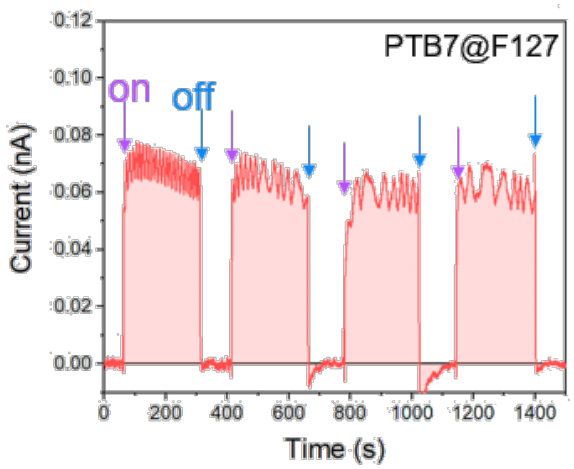


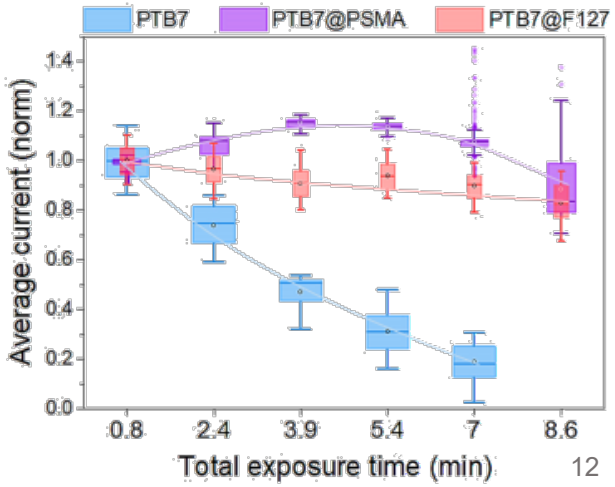
SOG production Quantum Yield

Ethanol	Water
52%	50%
8.5%	32.4%
0%	0.38%

Superoxide radical







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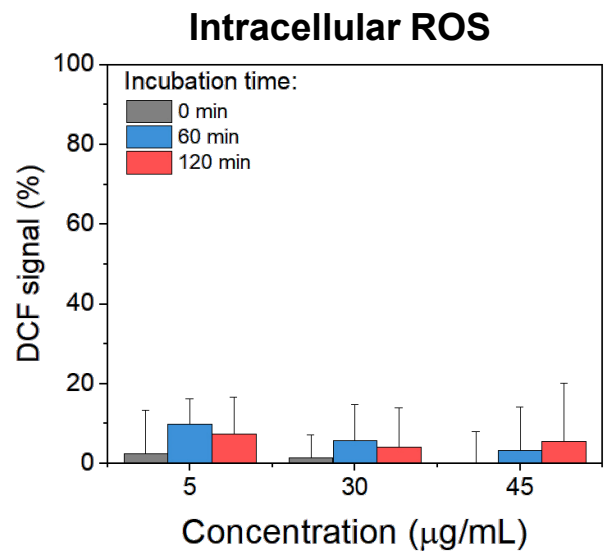
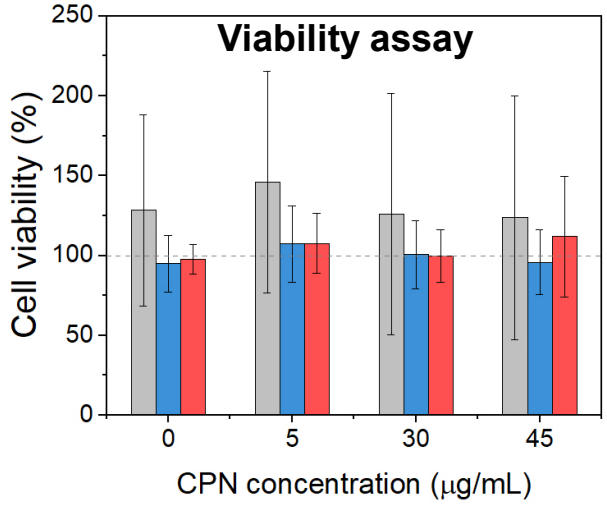
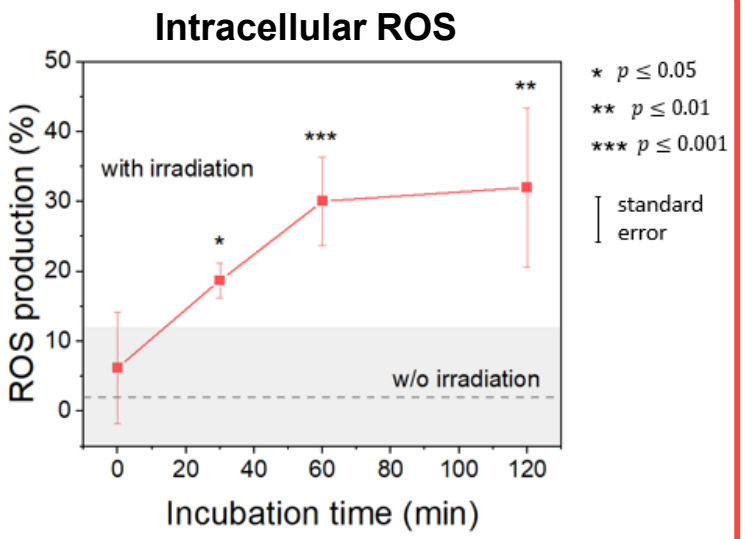
In vitro assays with PTB7@F127 CPNs on cancerous cells

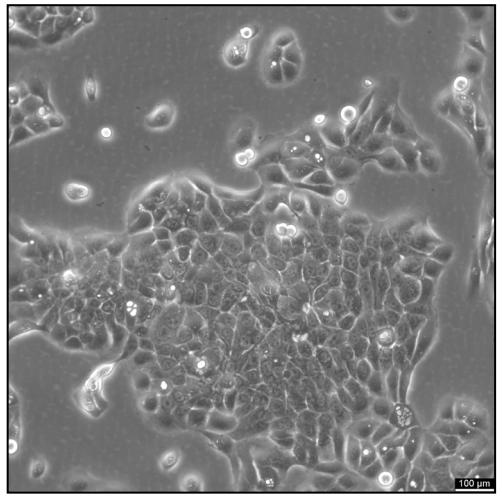
In the dark





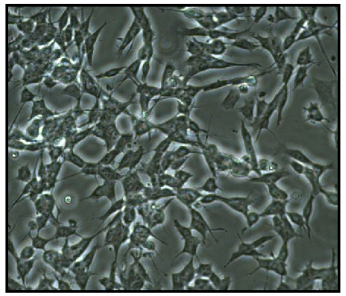
Under NIR illumination



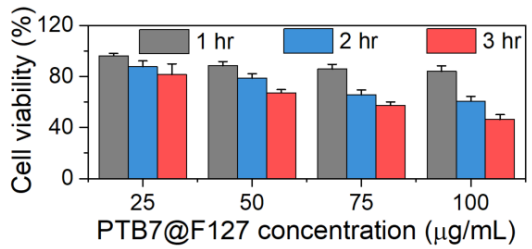


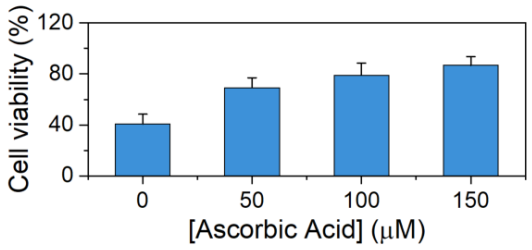
HaCaT cell line

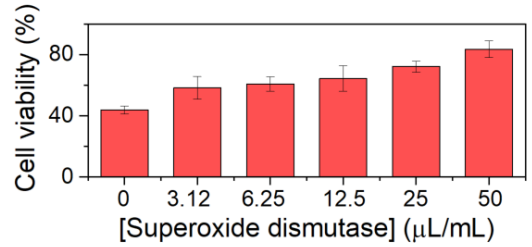
Dose & oxidative controls



SH-SY5Y neuroblastoma cells







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PTB7-based probes as bioimaging agents *in vitro*

A549 cell line

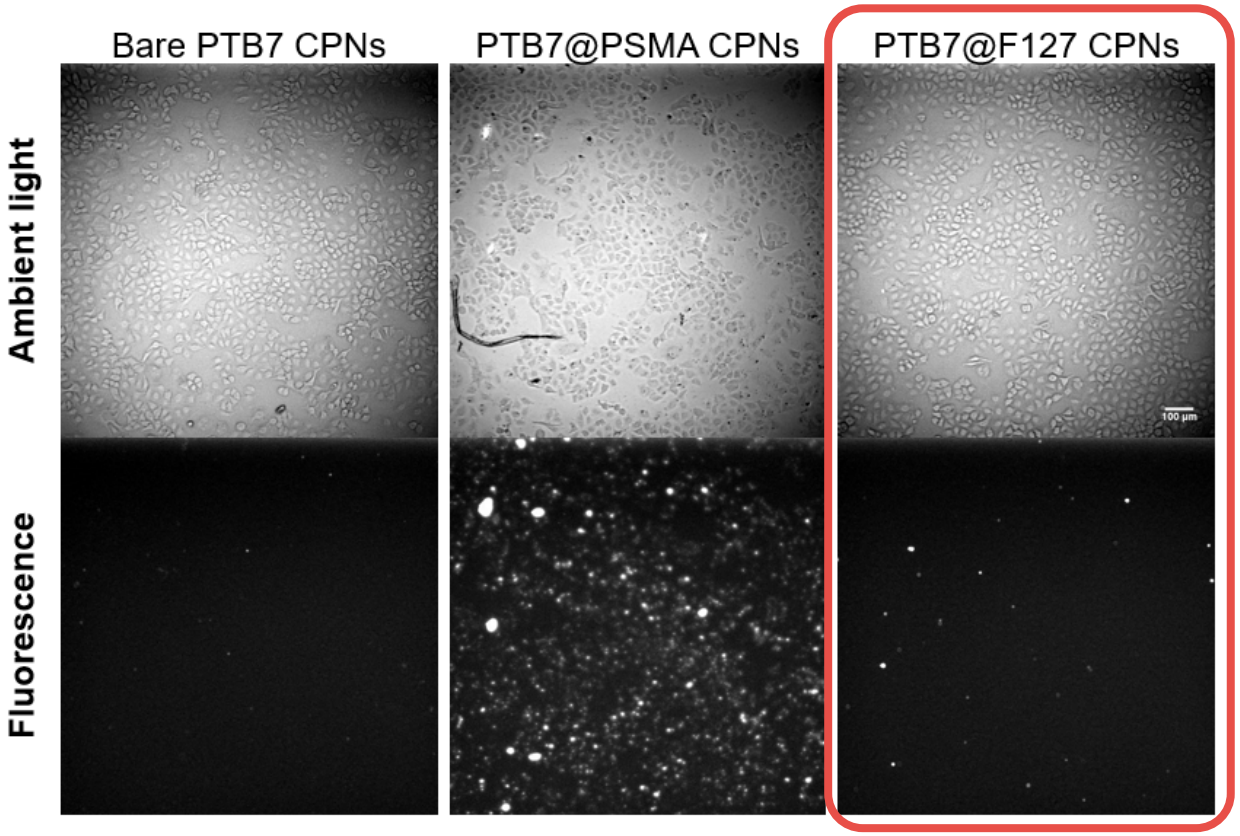
Incubated with CPNs overnight

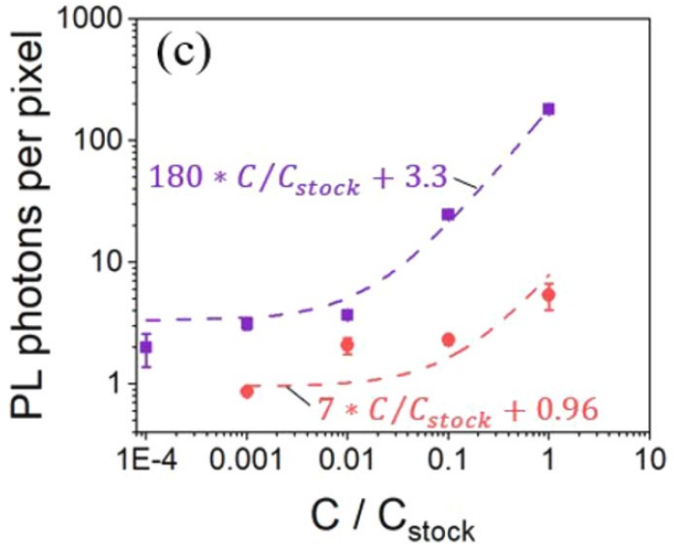
Washed, fixed and then imaged

400-640 nm excitation (filtered microscope lamp)

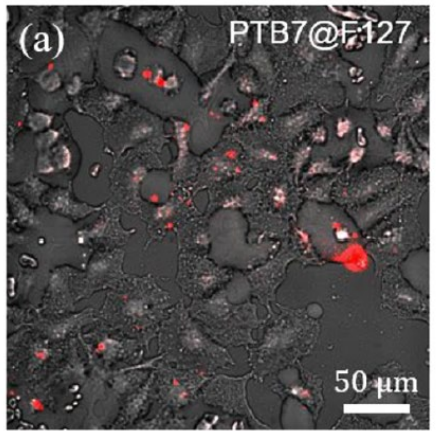
700-1000 nm collection window

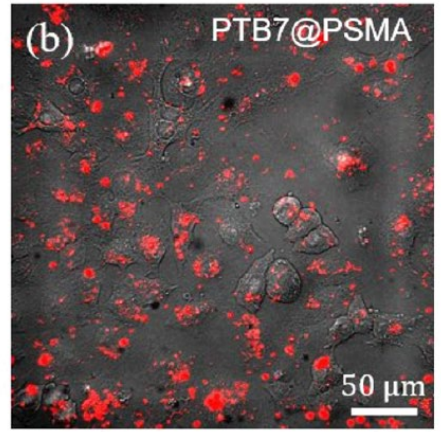
Specificity?





Superimposed BF and PL images





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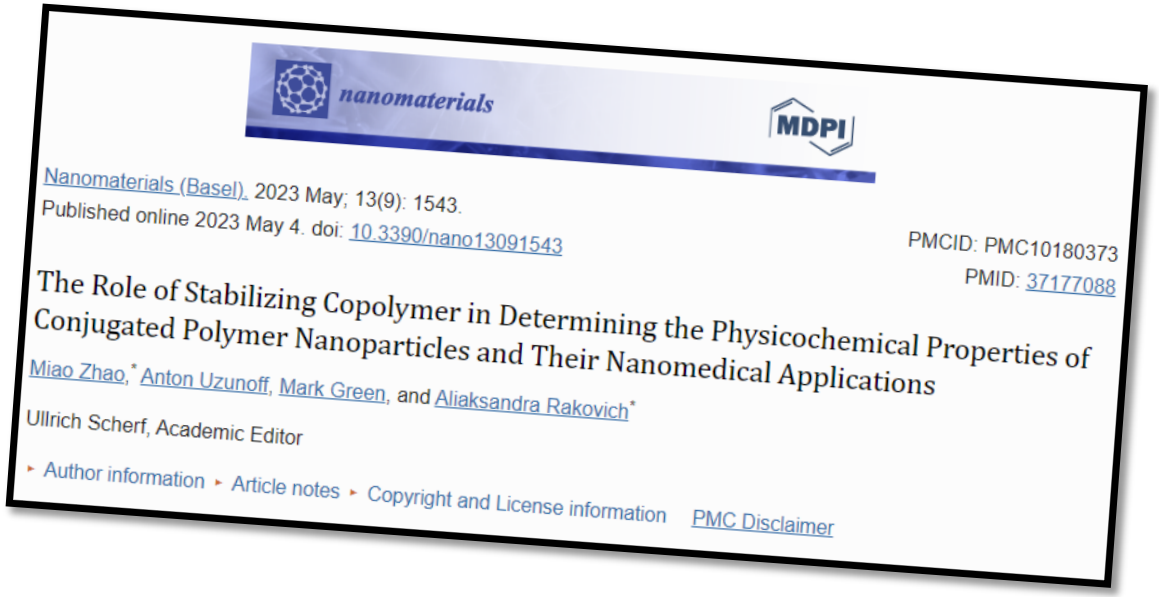
Conclusions

PTB7 CPN-based theranostic probes

- NIR-active – minimizes tissue absorption
- Appropriate size
- Stable *ex vivo, in vivo* and under physiological conditions
- Low dark cytotoxicity
- Produce ROS under photo-excitation
- Can be functionalized to endue specificity to tumours
- Probe properties depend on *stabilizing shell*!
- **F127-stabilized PTB7 CPNs particularly promising**



nanomaterials

MDPI

Nanomaterials (Basel). 2023 May; 13(9): 1543.
Published online 2023 May 4. doi: 10.3390/nano13091543

PMCID: PMC10180373
PMID: 37177088

The Role of Stabilizing Copolymer in Determining the Physicochemical Properties of Conjugated Polymer Nanoparticles and Their Nanomedical Applications

Miao Zhao,* Anton Uzunoff, Mark Green, and Aliaksandra Rakovich*

Ullrich Scherf, Academic Editor

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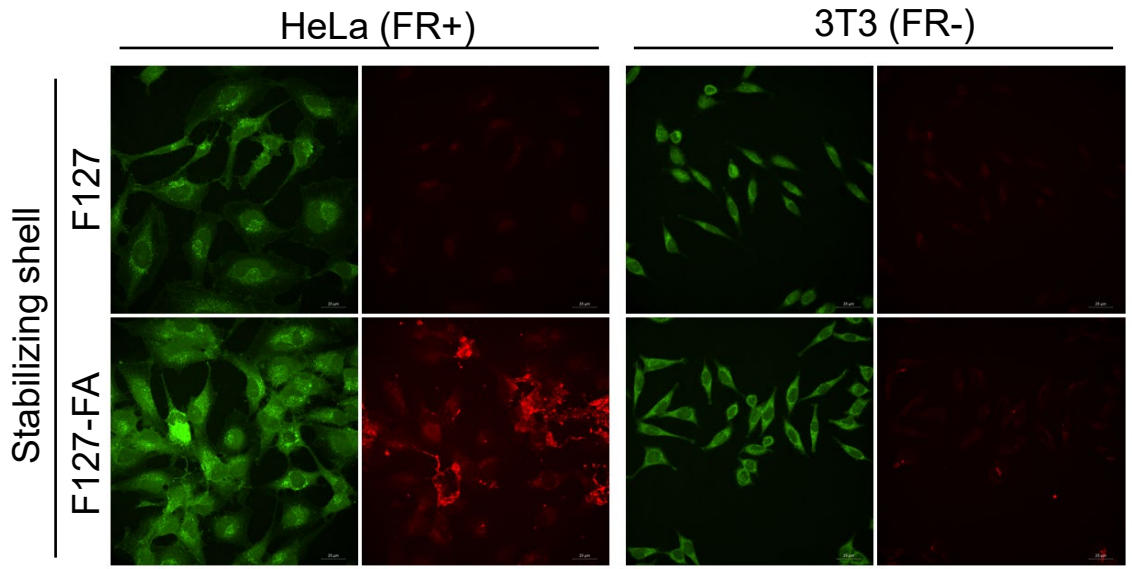

Current / future work

- Adding targeting capability to PTB&@F127 CPN (Miao, *in preparation*)
- Optically separating the imaging and photosensitizing modalities (Anton)

Acknowledgements

People:

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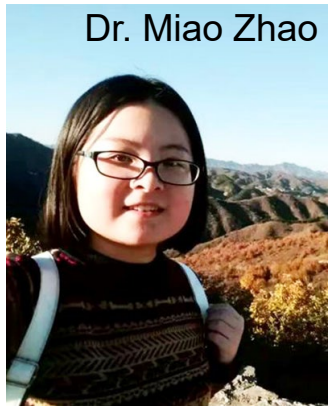

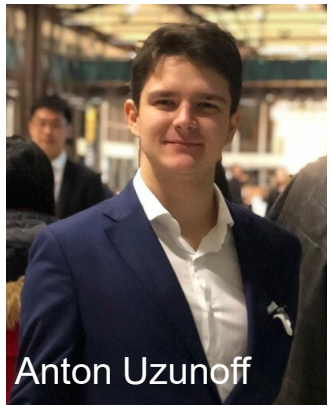

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Dr. Dave Simpson – CEO, Iksuda Therapeutics
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