Quantum Dot Conjugates for Imaging Applications

Sungjee Kim
Dept. of Chemistry
POSTECH

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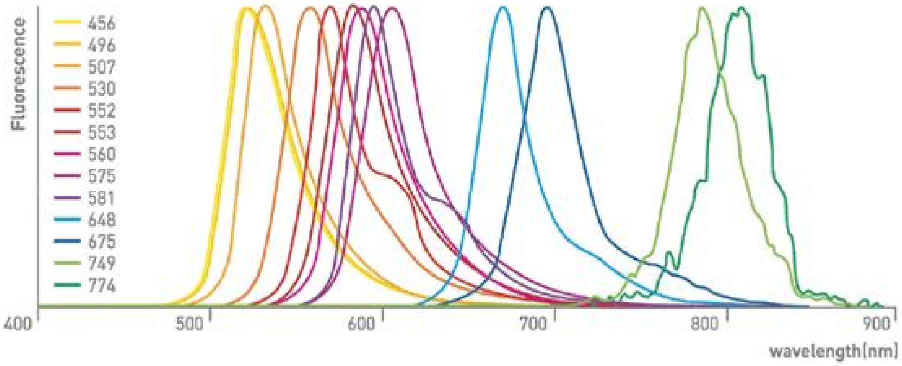
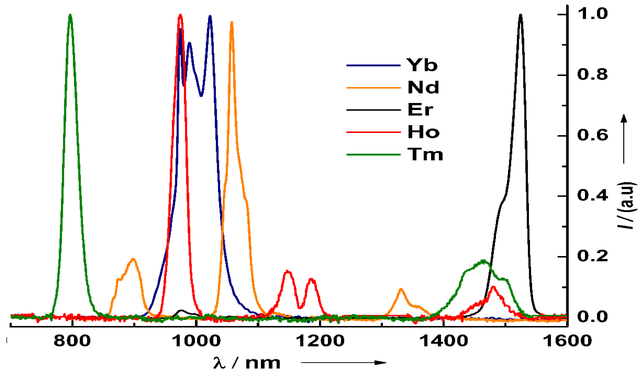
QD as Bright & Tunable IR Emitter



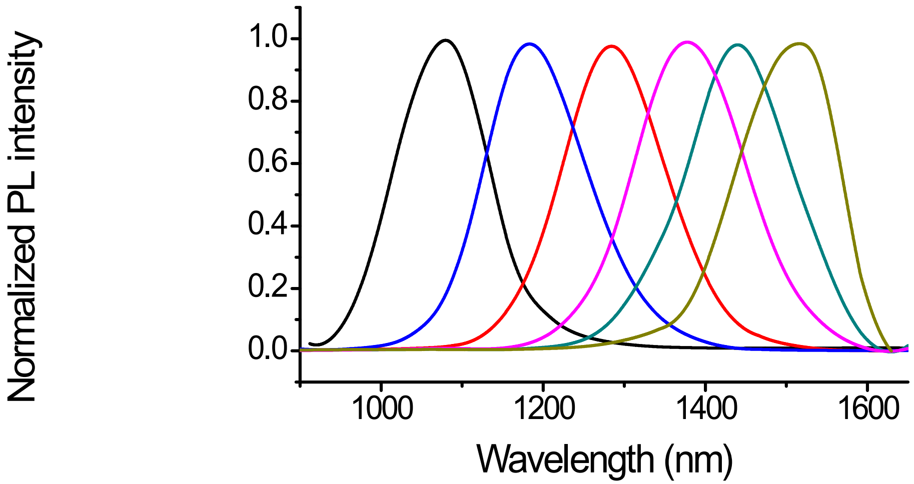
Organic dye molecules[1]

- Low quantum yield at NIR & IR range because of molecular vibration modes



Lanthanide complexes[2]

- Limited emission wavelength tunability
- Small absorption cross-section

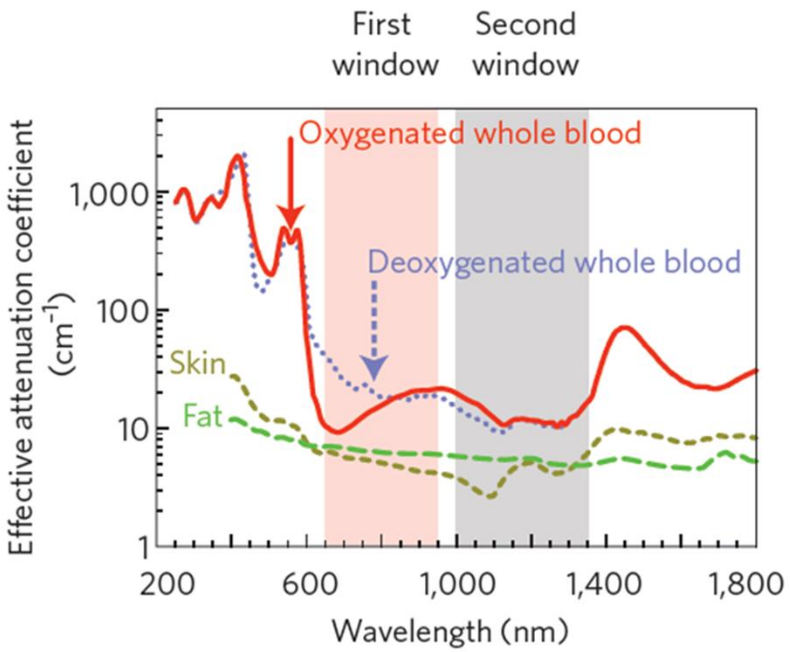


Quantum Dots at NIR & IR

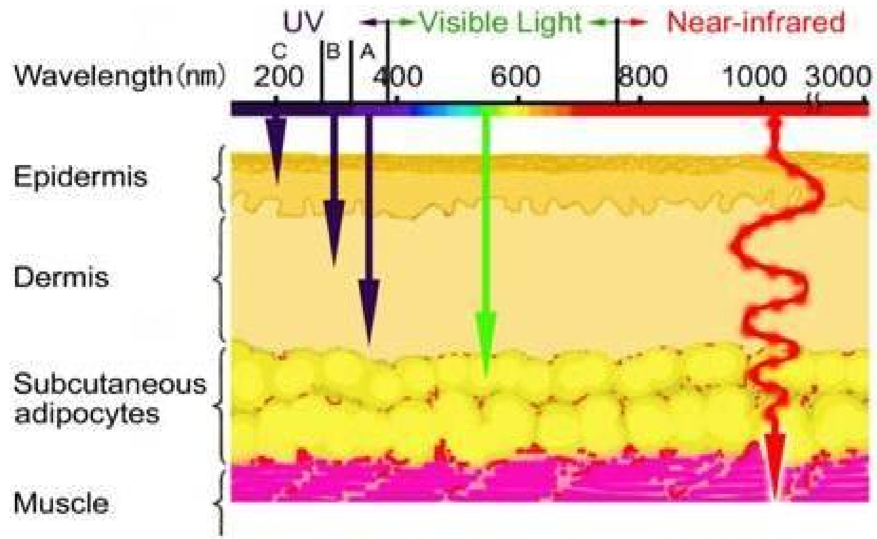
Bright and wavelength-tunable nano-emitters

[1] http://www.komabiotech.com.
[2] *Angew. Chem. Int. Ed.* **2005,** *44*, 2508.
Quantum dot emission spectra: *unpublished data*

Advantage of Near-infrared Region Imaging



<Effective attenuation coefficients of biomolecules>



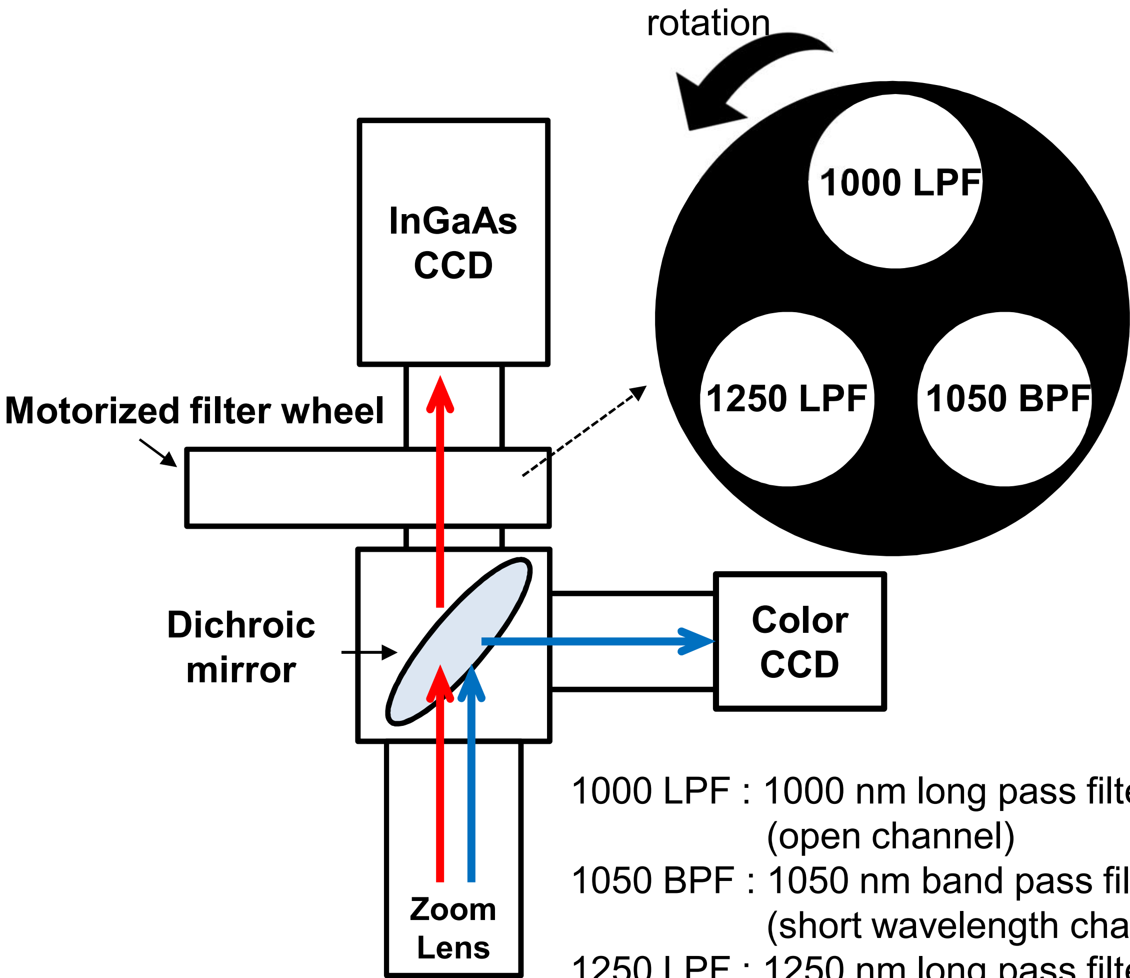
<Tissue penetration depth of lights>

- **Biomolecules have <u>lower absorption</u> and <u>scattering</u> in the NIR region.**
- **The NIR optical window can maximize the tissue <u>penetration depth</u>.**

First optical window (FOW; 700 – 900 nm)
Second optical window (SOW; 1000 – 1400 nm)

Nat. Nanotechnol. **2009**, *4*, 710

Imaging Setup for NIR Fluorescence Multiplexed Imaging



1000 LPF : 1000 nm long pass filter (open channel)
1050 BPF : 1050 nm band pass filter (short wavelength channel)
1250 LPF : 1250 nm long pass filter (long wavelength channel)

blue arrow: visible light
red arrow: infrared light

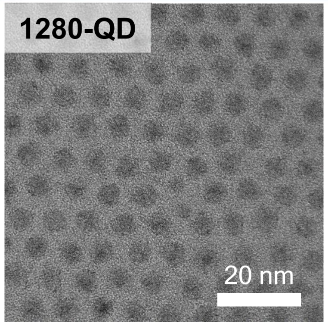
Adv. Healthcare Mater. **2018**, 7, 1800695.

PbS/CdS QDs for Multiplexed Imaging

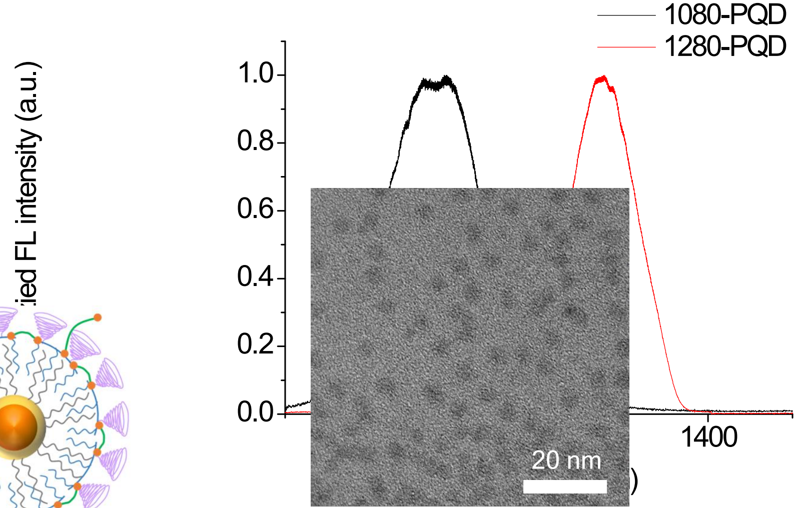
M images PbS/CdS QDs for multiplexed imaging

1080-QD

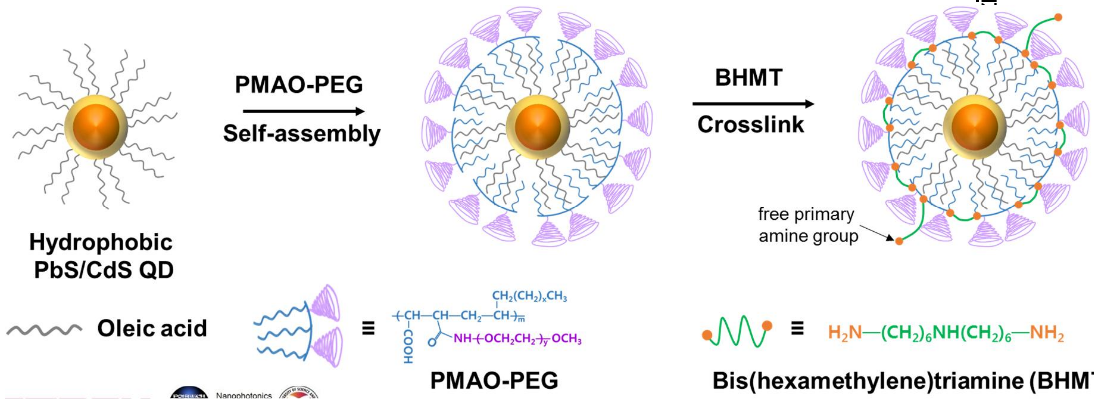
1280-QD



Normalized FL spectra of two PQDs



Fabrication of polymer-encapsulated QDs (PQDs)



AO-PEG : poly(maleic anhydride-alt-1-octadecene) conjugated with poly(ethylene glycol)

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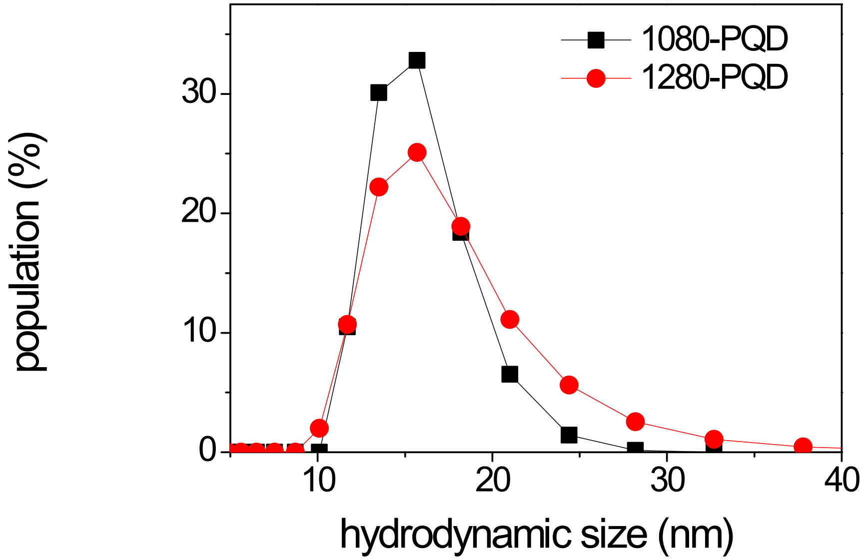
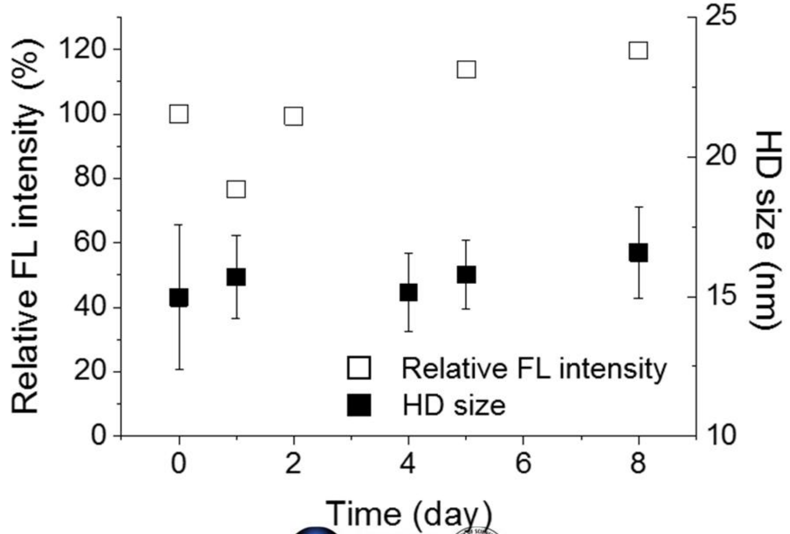
Polymer-encapsulated QDs (PQDs)



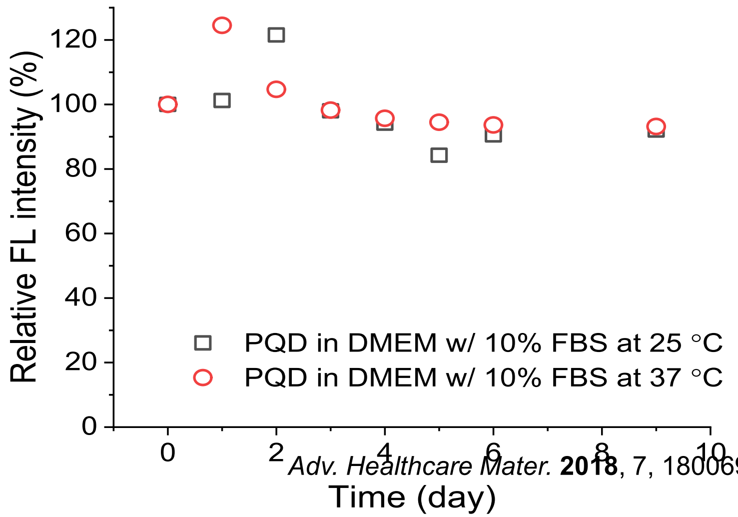
Dynamic light scattering histogram of the hydrodynamic (HD) size of polymer-encapsulated QDs (PQDs).

1080-PQD and 1280PQD show the same hydrodynamic size and the same Zeta potential.

Relative FL intensity and HD size change over time for PQDs in water



Relative FL intensity change over time for PQDs in cell growth media



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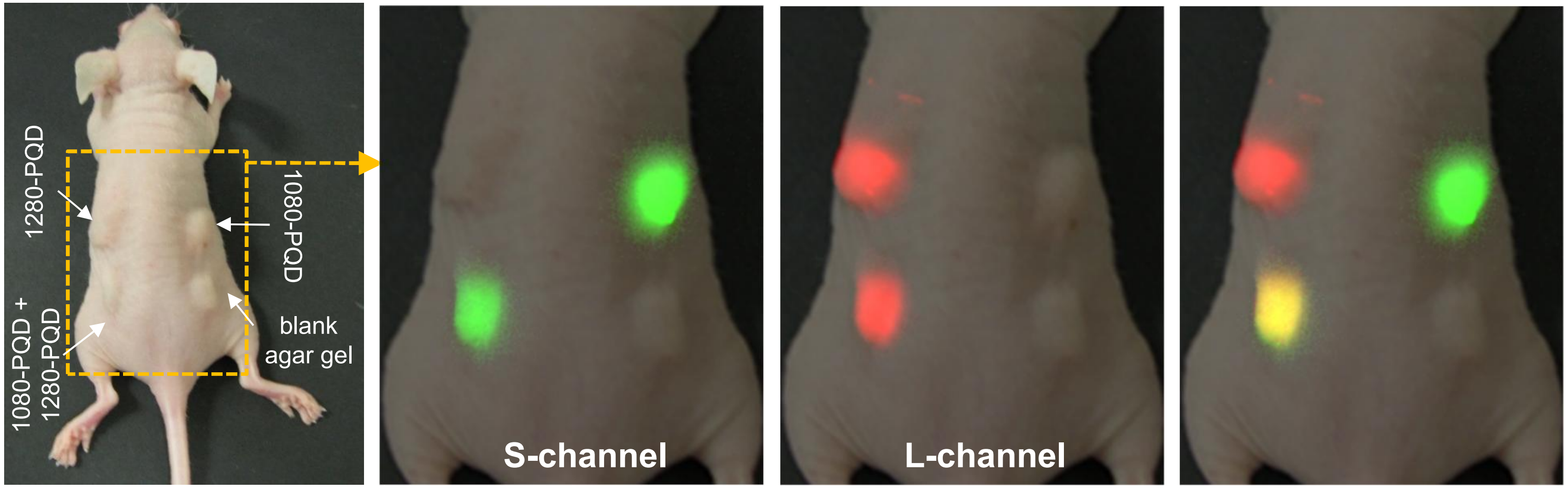
NIR Fluorescence Multiplex Imaging

PQD aqueous solutions

Nude mouse that was subcutaneously injected agar gel-PQD mixtures



S-channel; 1050 nm band pass filter
L-channel; 1250 nm long pass filter
O-channel; 1000 nm long pass filter

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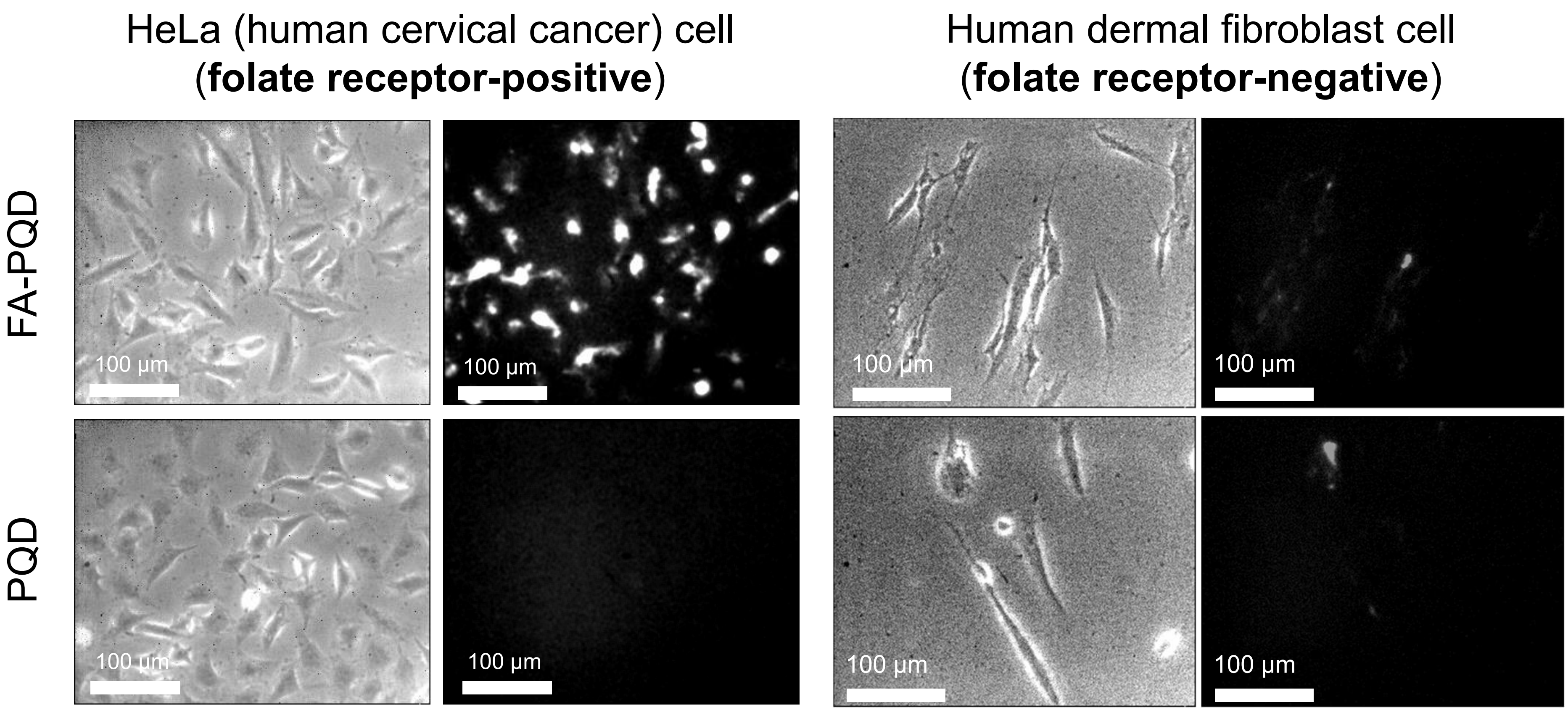
Bioconjugation of PQDs



- 300 nM FA-PQDs or unconjugated PQDs were co-incubated with cells for 8 h.
- FA-PQDs can specifically target and label cancer cells that overexpress folate receptors.

PQD : polymer-encapsulated QD
FA-PQD : folic acid-conjugated PQD

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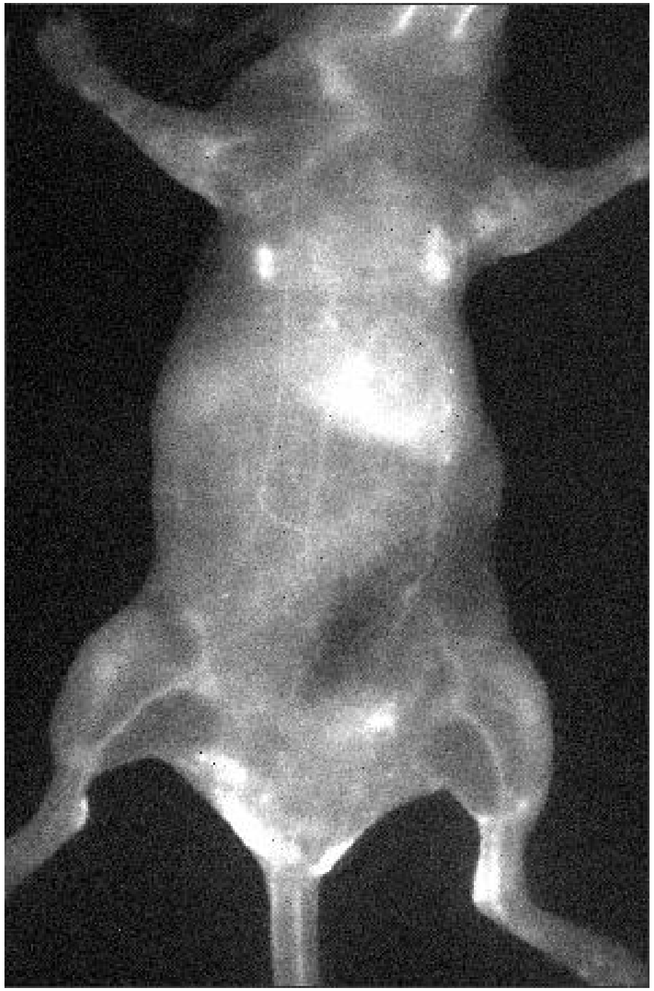
Whole body *in vivo* NIR-II image

The mouse was intravenously injected with a mixture of two color NIR-II probes: 1080-PQD and folic acid-conjugated 1280-PQD (FA-1280-PQD). NIR-II FL images under L-channel for FA-1280-PQD signals. The FL images were taken 5 min after the injection.



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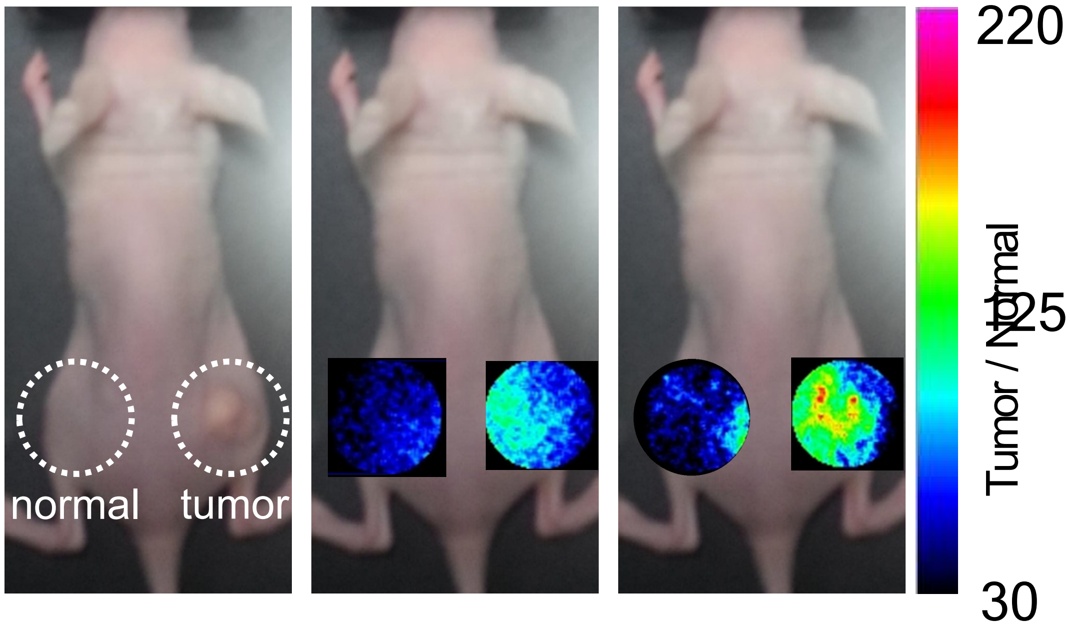
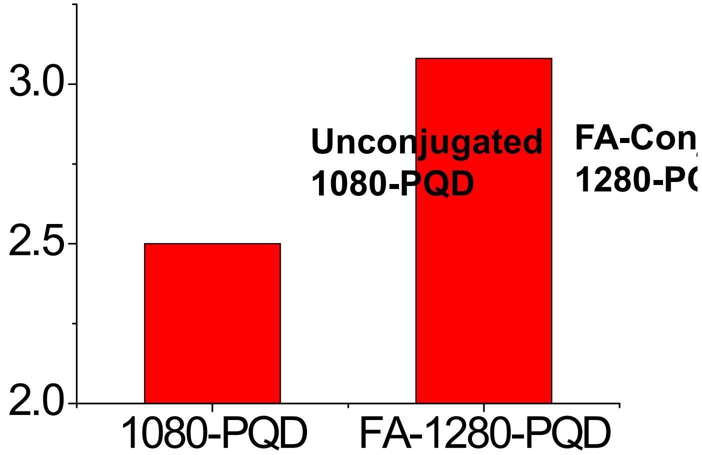
In vivo Multiplexed NIR-II Imaging

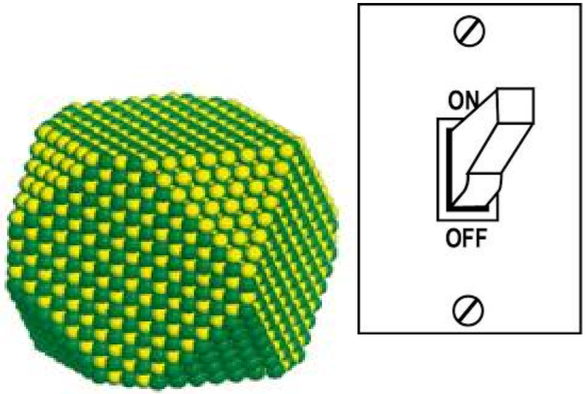


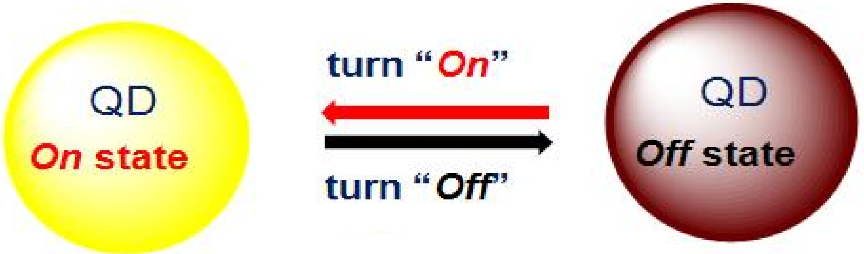


(taken 140 min after the inject

- This NIR-II whole body imaging with t ligand-assisted tumor-targeting of the permeation and retention effects in tu hydrodynamic size and surface prope

Switching Quantum Dot (QD) Fluorescence





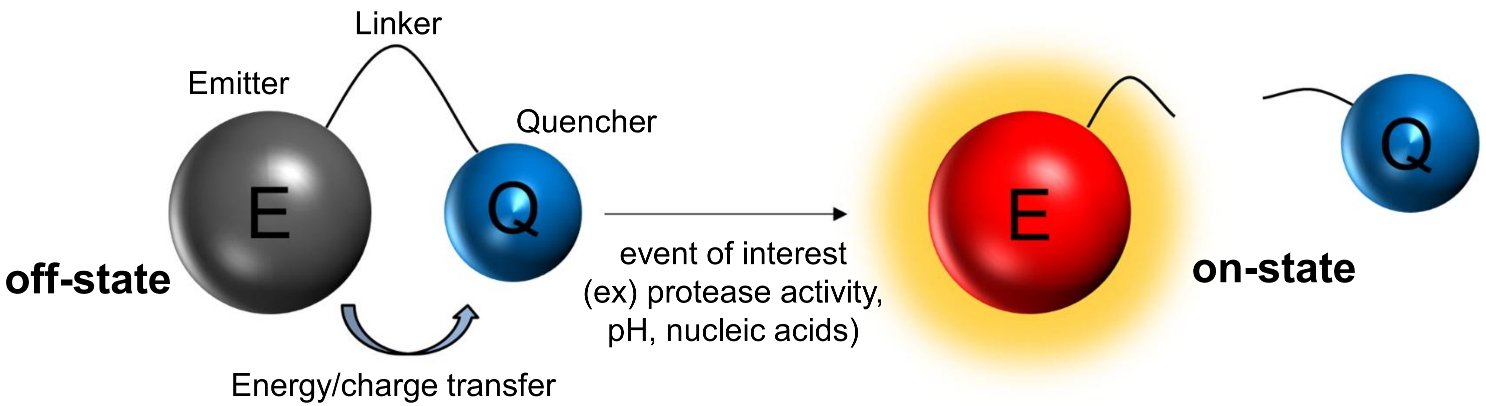
Attaching a switch onto a QD, thus making the QD-Switch conjugate can be turned on and off responding to external stimuli: light, analyte concentrations, (pH, ions, etc), enzymatic activities, and binding events (small molecule or antigen binding).

Applications for sensors, in vivo probes, imaging, memory, etc.

Activatable fluorescent probes

Activatable fluorescent probe : fluorophore whose signal is amplified by the biological event of interests such as enzymatic activity, pH, nucleic acids

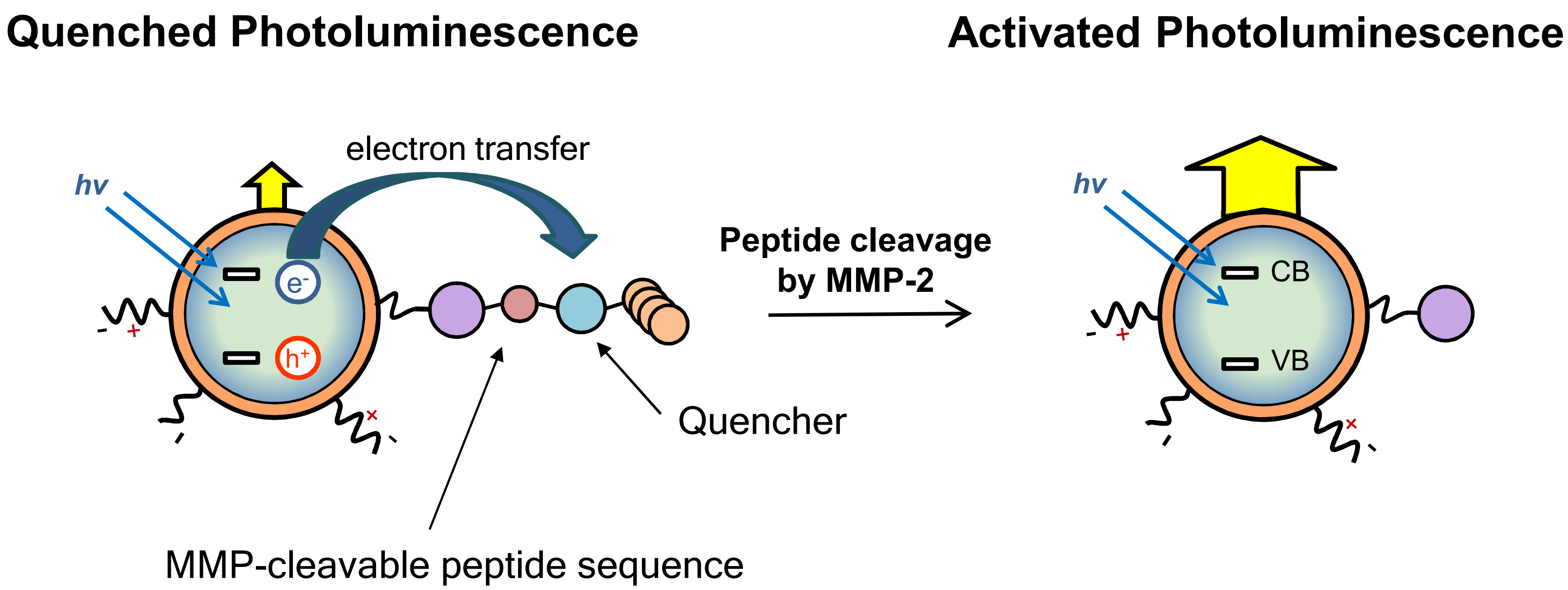
Simple scheme of activatable fluorescent probe



- Sensitive detection of protein activity, nucleic acid, pH in *in vitro* and *in vivo* with low background signal
- Activatable NIR-II QDs were not reported yet

Bremer, C.; Tung, C. H.; Weissleder, R. *Nat. Med.* **2001**, *7*, 743.
Lee, S.; Park, K.; Kim, K.; Choi, K.; Kwon, I. C. *Chem. Commun.* **2008**, 4250.

Design of Matrix Metalloproteinase(MMP)-activatable probe for cancer-microenvironment detection



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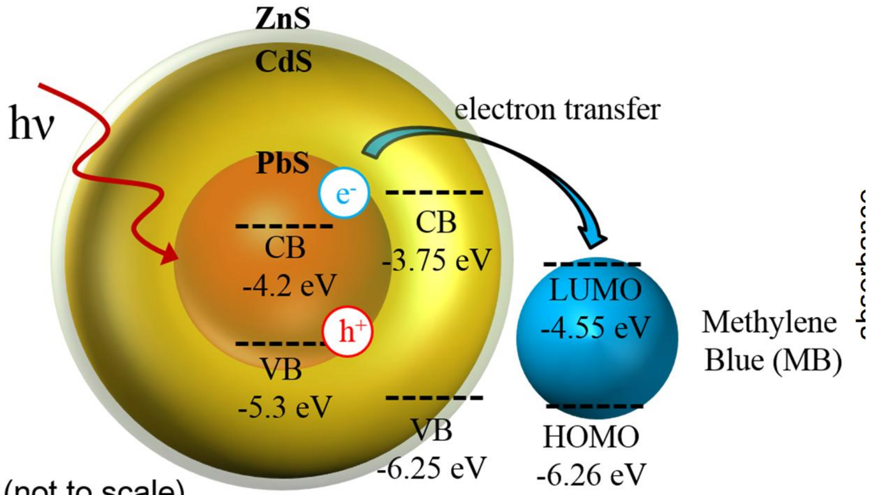
Quenching via photoinduced electron transfer by methylene blue

Methylene blue (MB) :

Energy level diagram of PbS/CdS/ZnS QD and MB

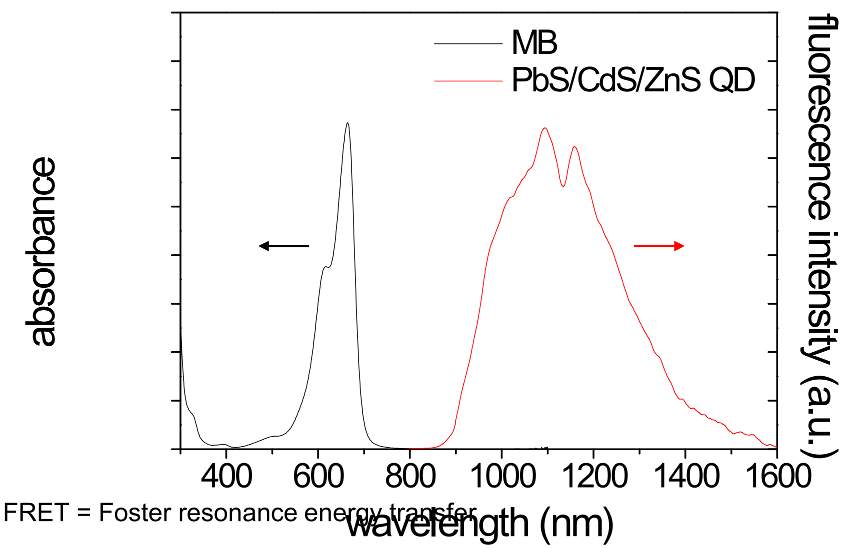


(not to scale)

Fluorescence quench via electron transfer was expected

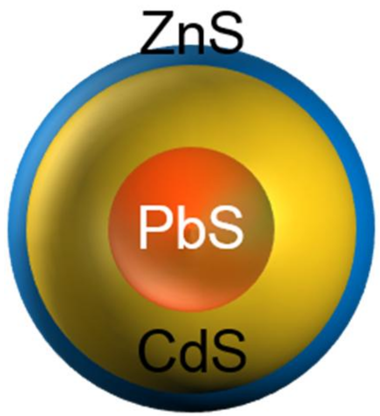
CB = conduction band
VB = valence band

Absorption spectrum of MB and fluorescence spectrum of QD

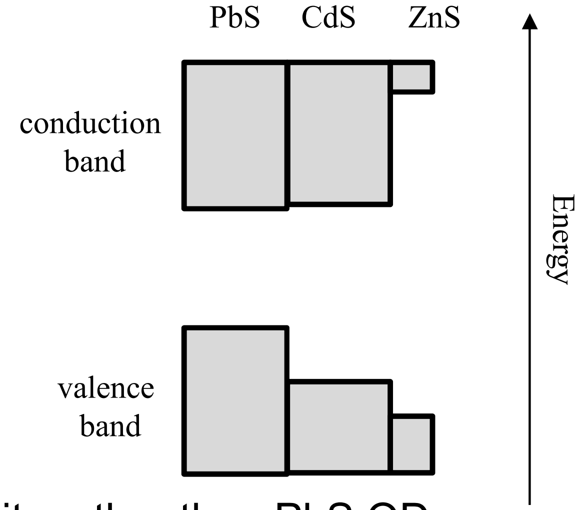


FRET = Foster resonance energy transfer

Synthesis of NIR-II emitting PbS/CdS/ZnS QD

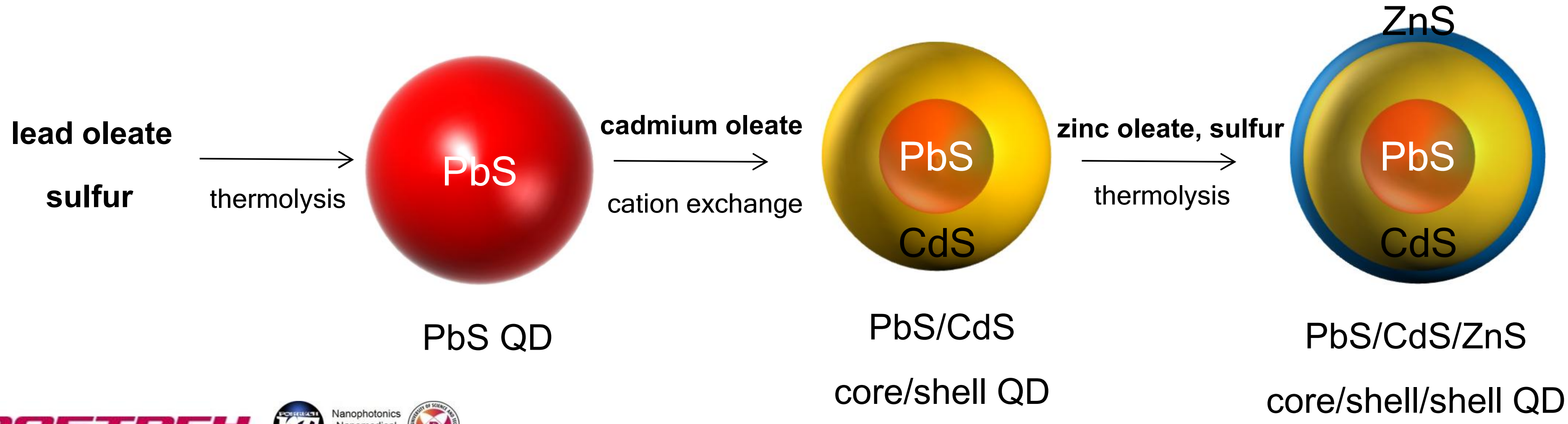


Energy level diagram of QD

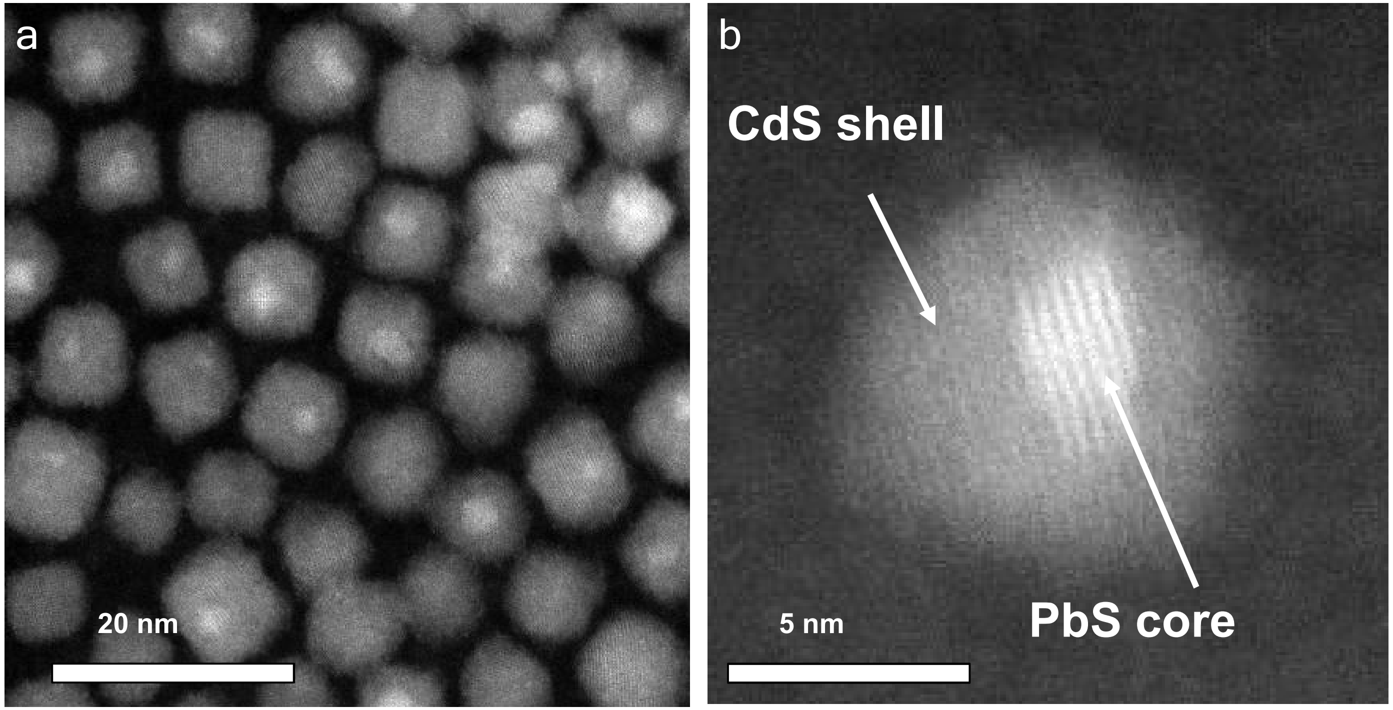


- Enhanced quantum yield and photostability rather than PbS QDs

Scheme for the fabrication of PbS/CdS/ZnS multishell QD



HAADF EM Image



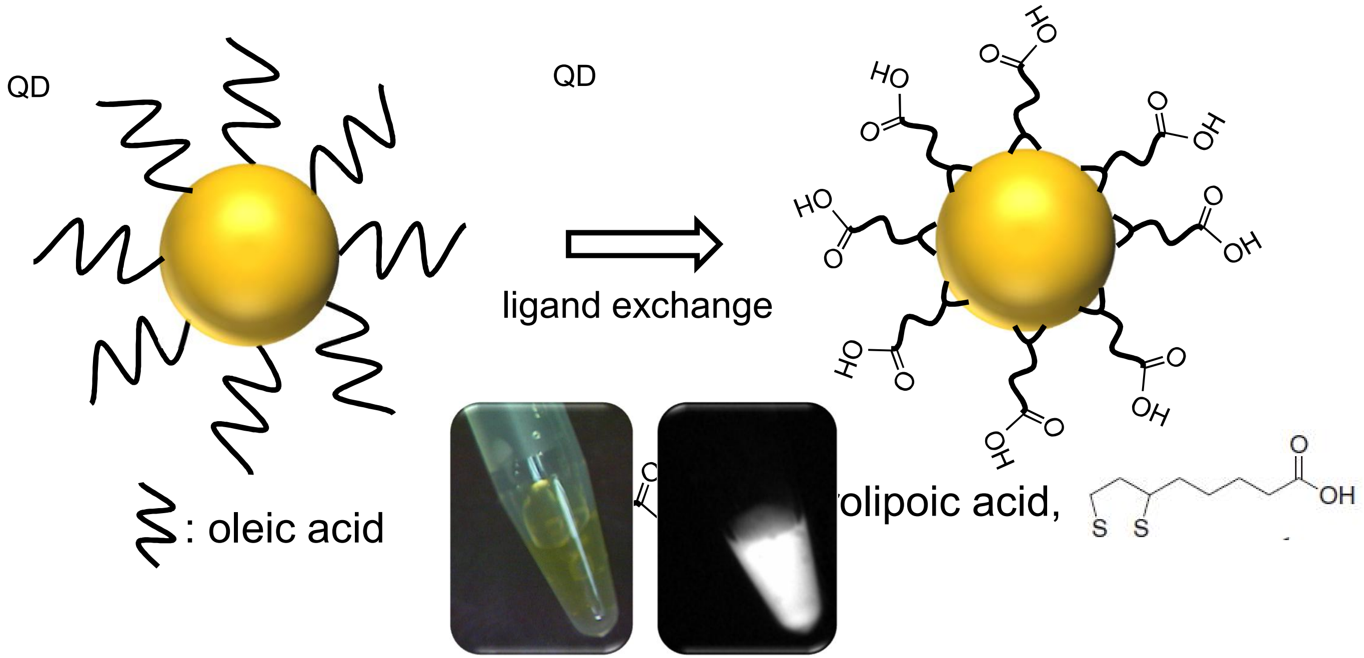
(a) STEM-HAADF image of PbS/CdS/ZnS QDs. (b) Magnified STEM-HAADF image of single PbS/CdS/ZnS QD.

STEM : Scanning transmission electron microscopy
HAADF : High-angle annular dark-field imaging

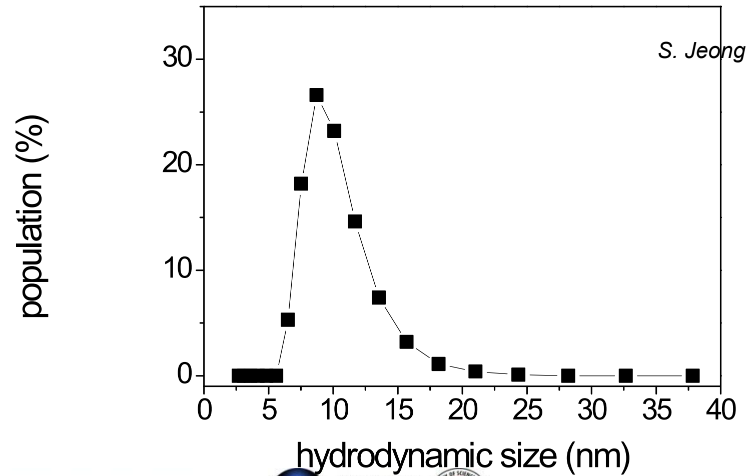
S. Jeong et. al. Nano Letters, **2017,** *17,* 1378−1386.

Water-soluble PbS/CdS/ZnS QDs

Ligand exchange from hydrophobic to hydrophilic QD



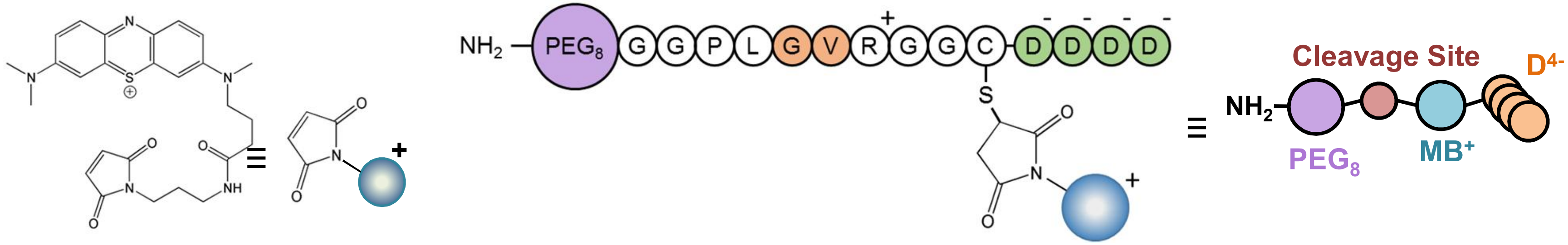
S. Jeong et. al. Nano Letters, **2017,** 17, 1378–1386.



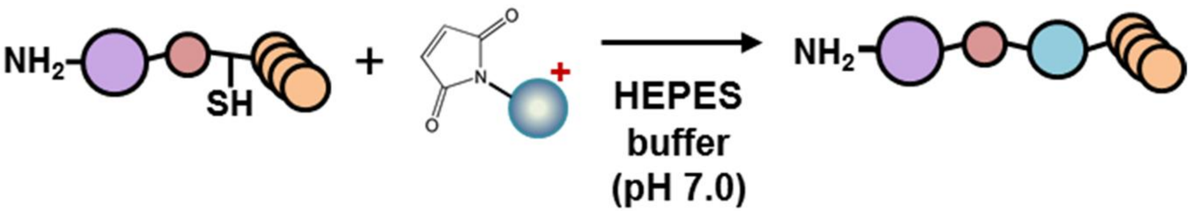
Surface modification for activatable probe

maleimide-MB

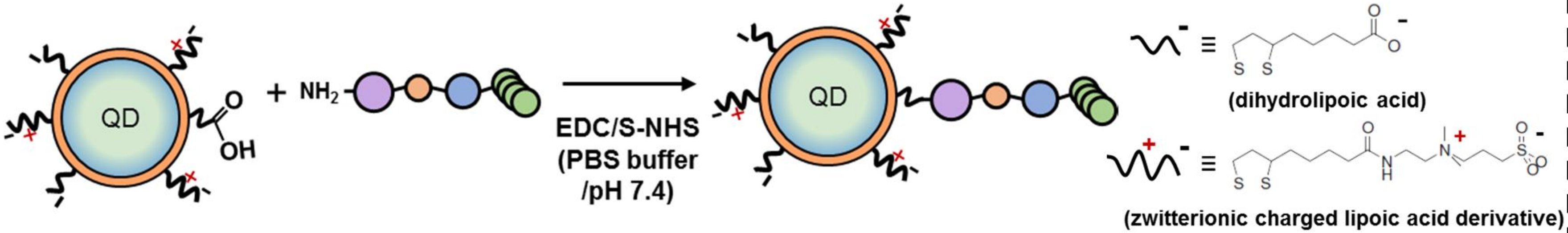
MMP-cleavable peptide sequence (MMCP)



Step 1: Maleimide coupling of methylene blue and MMPCP



Step 2: Conjugation of MMPCP-MB with QD



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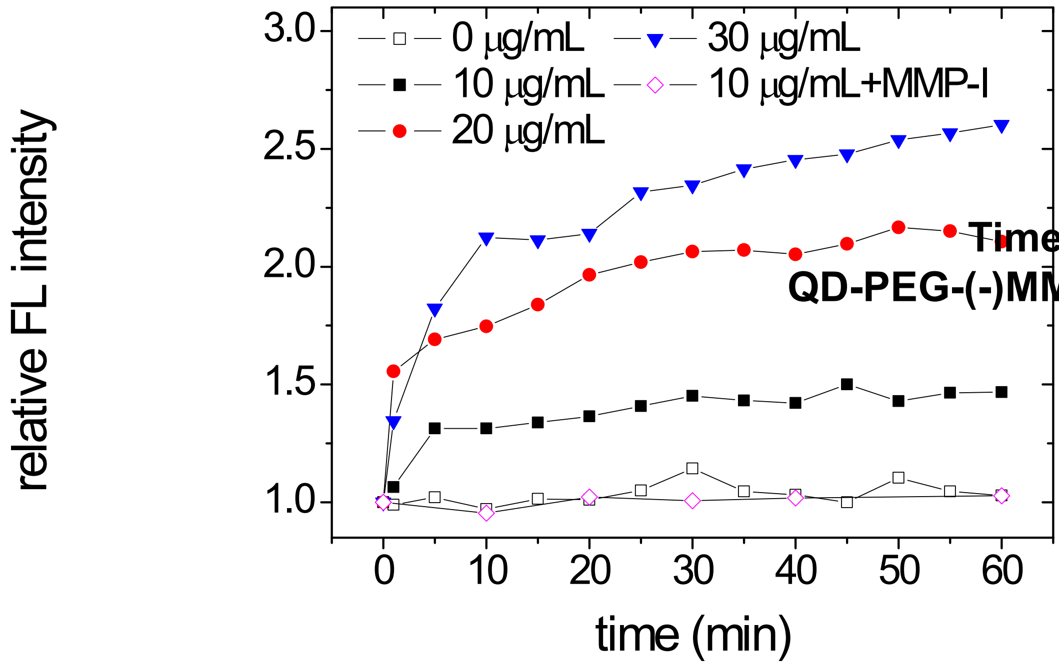
Quenching and activation of QD-MMPCP-MB complex



Time-dependent FL recovery of QD-PEG-(-)MMPCP-MB with MMP-2 concentration

100 nM QD-(-)MMPCP-MB solution
([MB]/[QD]=40)

buffer condition : 20 mM Tris, 0.1 mM Ca(NO
20 µM $Zn(NO_3)_2$, 100 mM NaCl
MMP-I : global MMP inhibitor

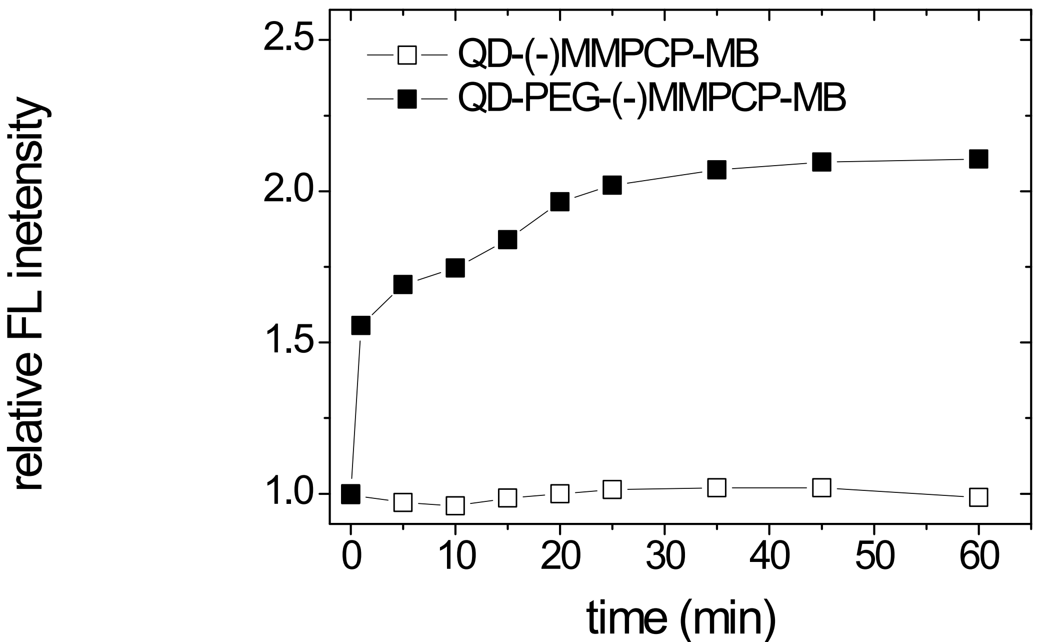
How to design the quencher peptide sequence
1. spacer sequence

QD-(-)MMPCP-MB

forbidden proteolysis by MMP-2

QD-PEG-(-)MMPCP-MB

allowed proteolysis by MMP-2



PEG = polyethylene glycol

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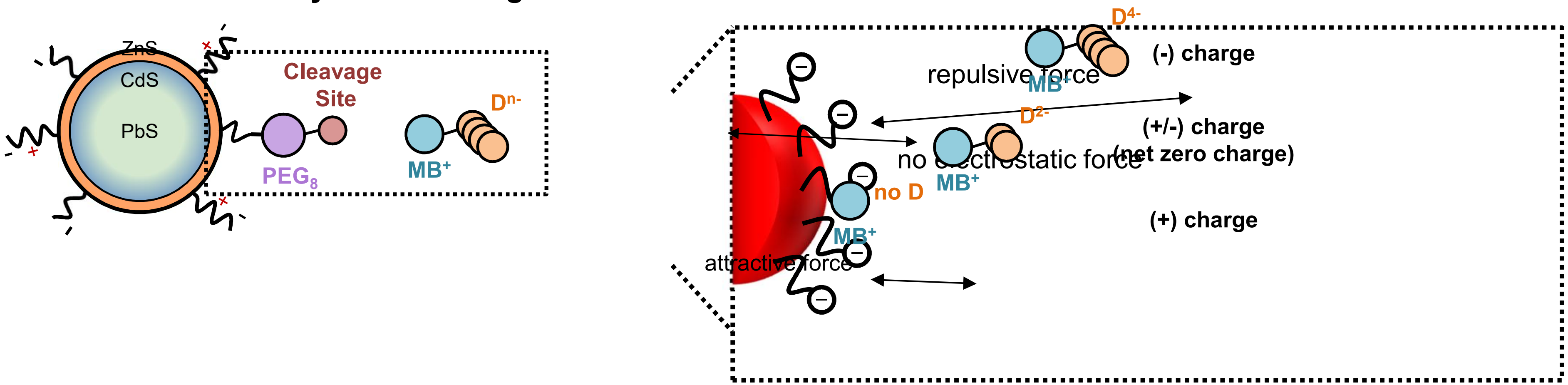
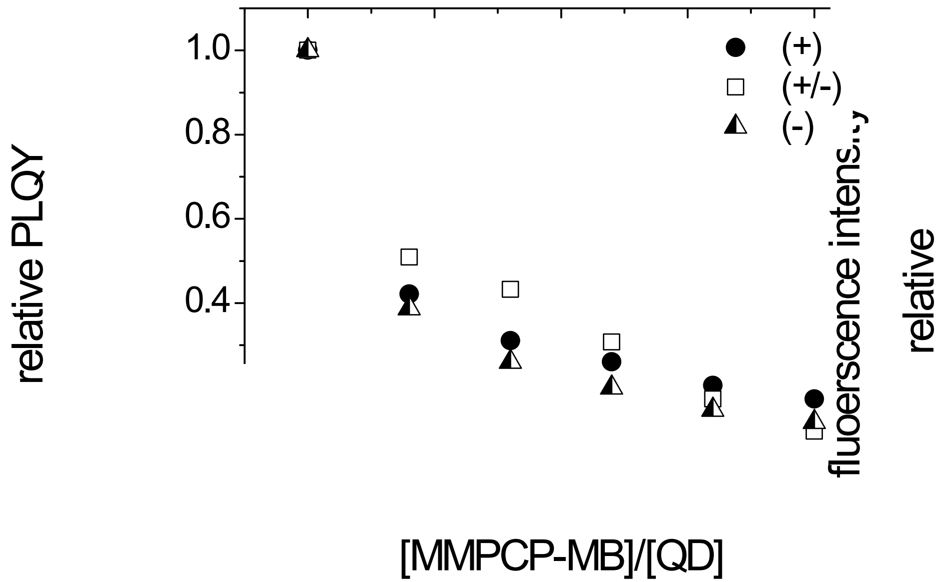
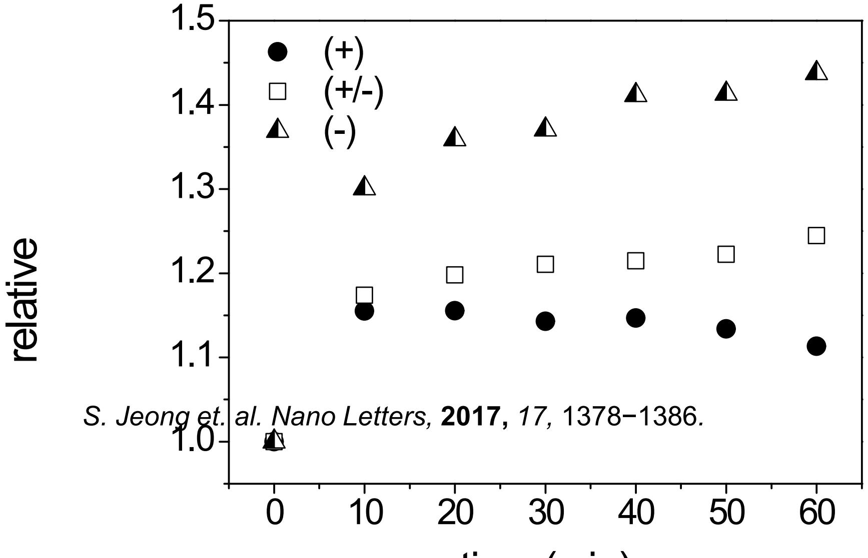
How to design the quencher peptide sequence
2. charged state of quencher sequence

After enzymatic cleavage





FL intensity of QD-MMPCP-MB



FL recovery of QD-MMPCP-MB with enzyme

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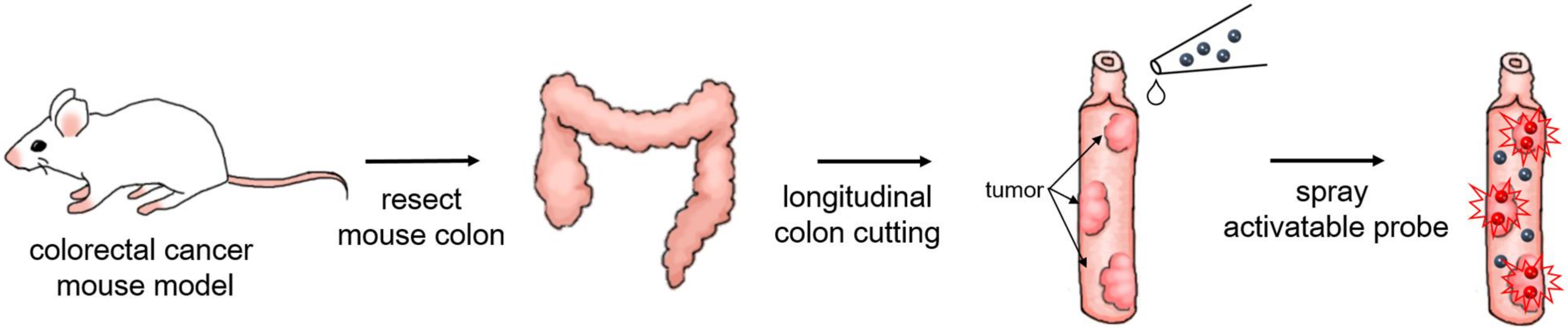
ex vivo fluorescence cancer imaging using NIR-II activatable probe

- colorectal cancer model (AOM/DSS-treated mouse) is known for high upregulation of MMPs in cancer microenvironment

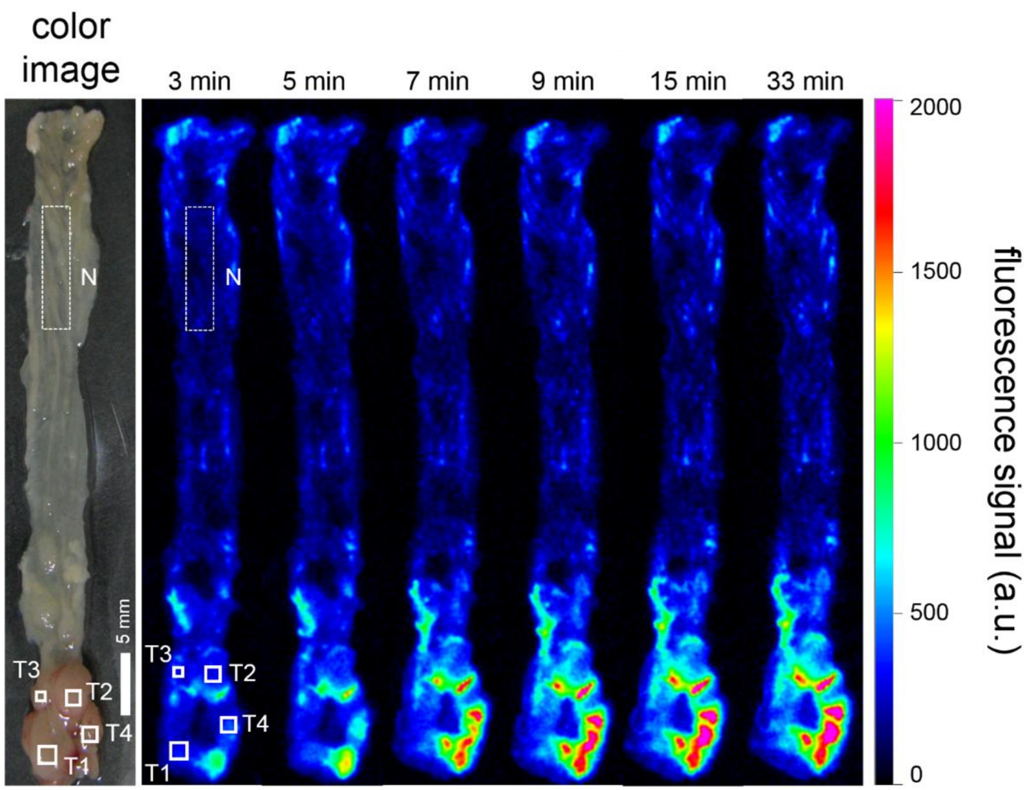
Scheme for *ex vivo* fluorescence imaging of colon cancer model



AOM : azoxymethane
DSS : dextran sulfate sodium salt

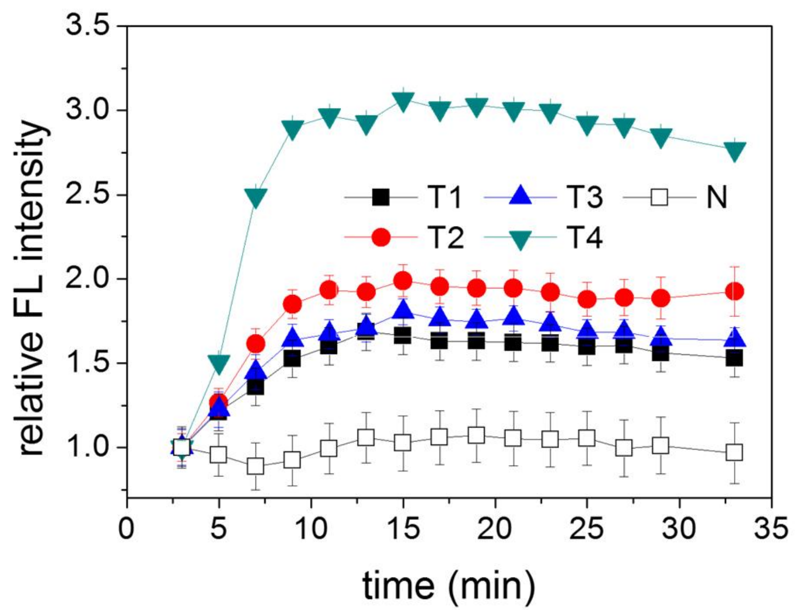
Colon cancer imaging with activatable probe

Time-dependent fluorescence image



Probe : 1 μM QD-PEG-(-)MMPCP-MB in PBS buffer at pH 7.4 ([MB]/[QD]=40)
excited by 910 nm laser with 200 mW/cm^2
exposure time = 90 ms

Time-dependent signal activation



Cancer microenvironment-specific fluorescence activation

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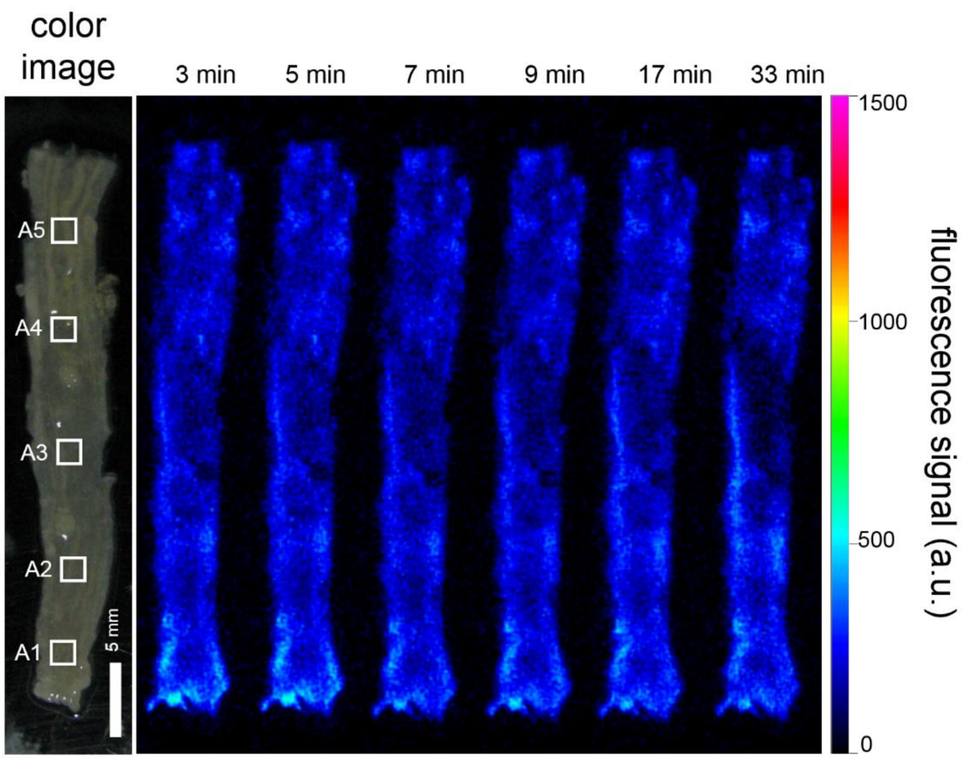
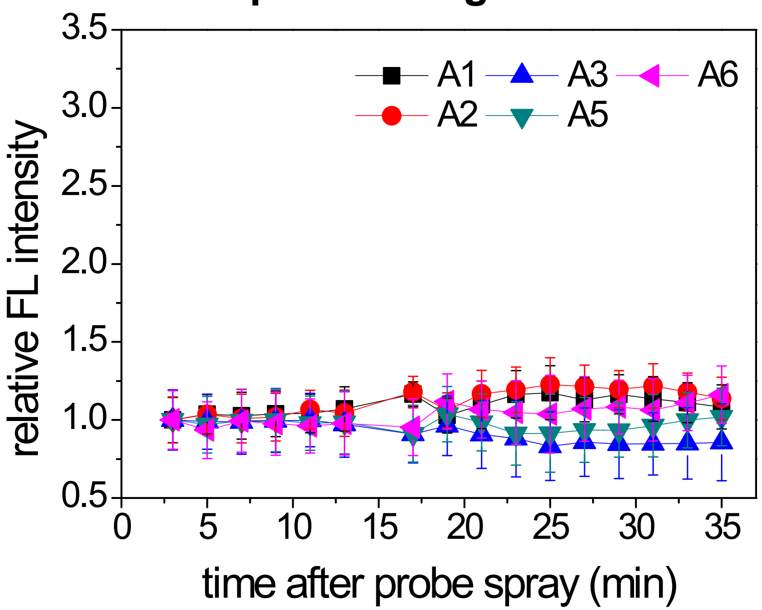
Normal colon imaging with activatable probes

Time-dependent fluorescence image





Probe : 1 μM QD-PEG-(-)MMPCP-MB in PBS buffer at pH 7.4 ([MB]/[QD]=40)
excited by 910 nm laser with 200 mW/cm^2
exposure time = 90 ms

No noticeable fluorescence activation

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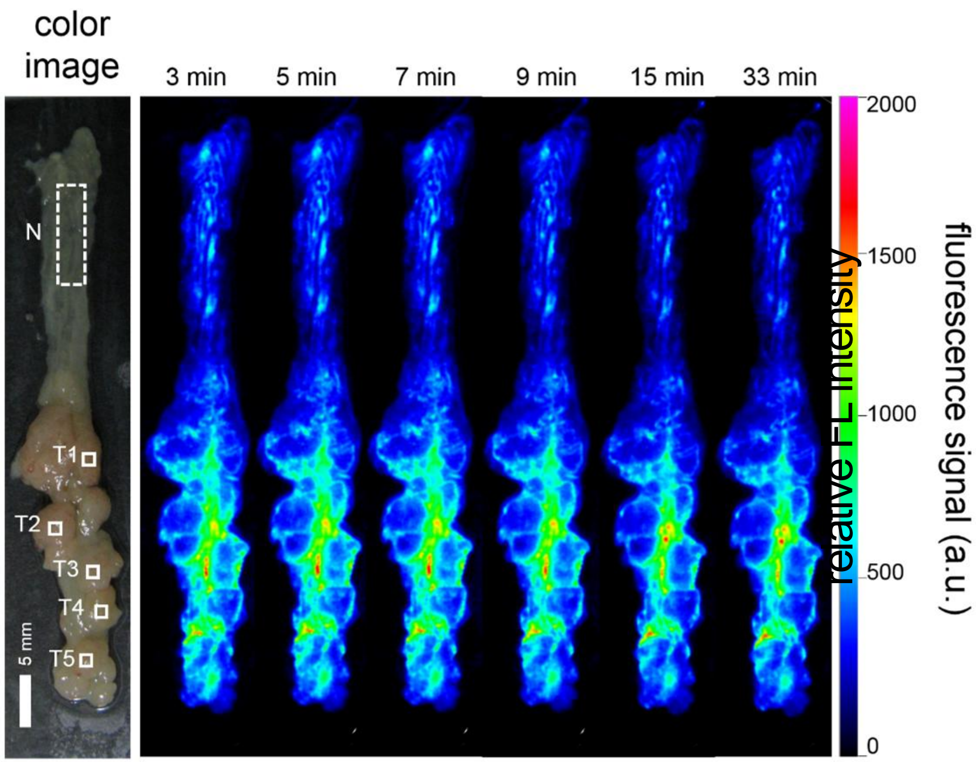
Colon cancer imaging with non-activatable probes

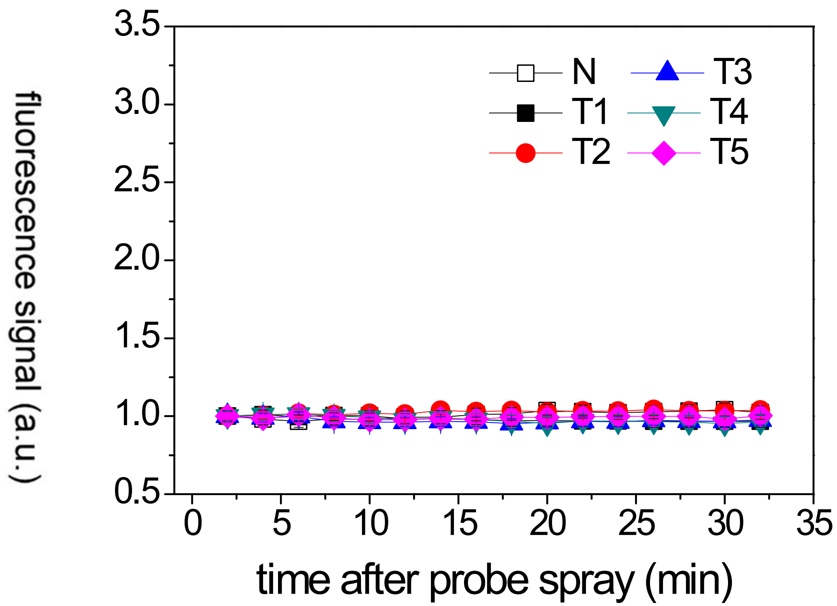
non-activatable probe = QD without MMPCP-MB

Time-dependent fluorescence image



Probe : 1 μM QD in PBS buffer at pH 7.4
excited by 910 nm laser with 200 mW/cm^2
exposure time = 90 ms

Time-dependent signal activation



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Collaborator (partial list):

Prof. Nam Ki Lee, SNU

Prof. Jong Bong Lee, POSTECH

Prof. Seung-Jae Myung, AMC

Prof. Chan Ki Pack, AMC

Prof. G-One Ahn, POSTECH

Prof. Junsang Doh, POSTECH

Prof. Jung-Joon Min, Chonnam Univ.

Prof. Chulhong Kim, POSTECH

Prof. Ki Hean Kim, POSTECH

Prof. Euiheon Chung, GIST

Thank you for listening.