Tunable Ultrasmall Visible-to- Extended Near-Infrared Emitting Silver Sulfide Quantum Dots for Integrin-Targeted Cancer Imaging

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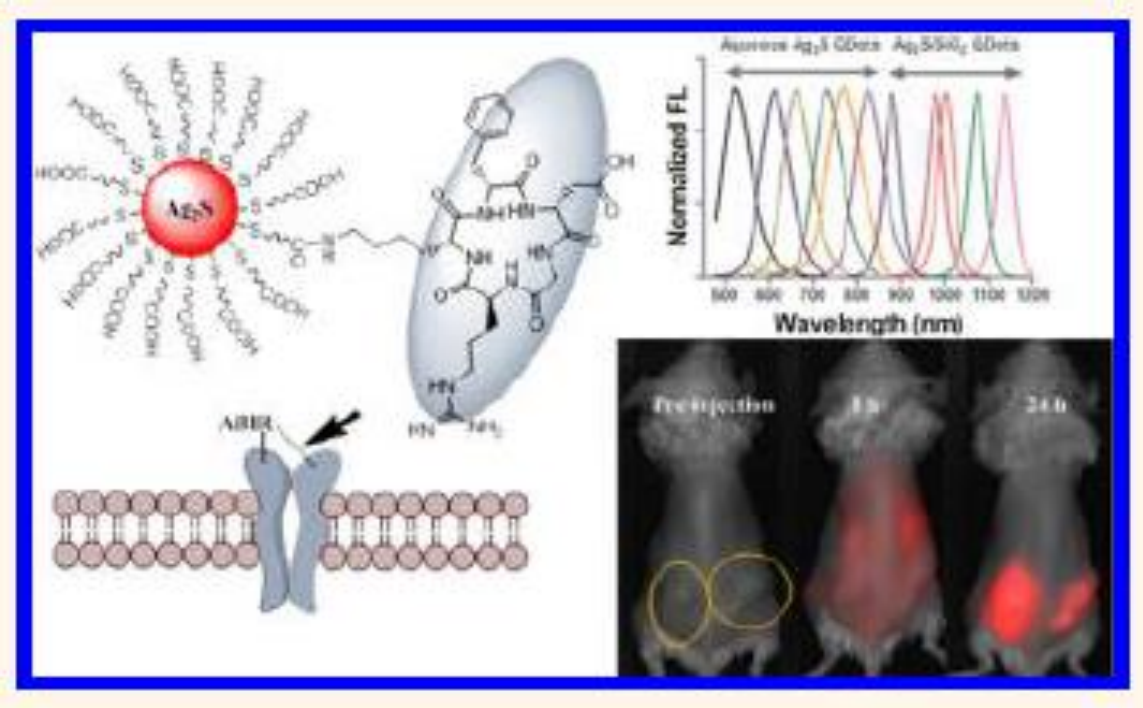

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Introduction

• Fluorescent nanoparticles, especially quantum dots (QDs), are widely used in biological imaging because their unique properties can overcome many drawbacks of conventional organic fluorescent dyes.

• These QD properties include broad absorption for ease of excitation at multiple wavelengths, narrow emission bands for multicolor imaging, high photostability for longitudinal imaging in cells and in vivo, and polyvalency for multifunctional applications.

• Although cadmium- and indium-based QDs exhibit the above desirable properties, concerns remain about the use of these nanomaterials in biological imaging applications.

• Polymer coated QDs have been successfully employed to lessen this potential toxicity.

• This approach significantly increase hydrodynamic diameter (>20 nm), but it also complicates the synthesis procedure and occasionally employs reagents that could induce systemic toxicity.

• The prolonged retention of large QDs in the vascular system induce cytotoxicity or reduce QD efficiency and sensitivity in imaging studies

• Luminescent silver sulfide (Ag_2S) nanoparticles within a narrow size range is of particular interest due its negligible toxicity in in vivo applications.

• However, a major challenge is the difficulty in synthesizing multiple Ag_2S QDs with distinct emissions in the nearinfrared (NIR) window (750 to 1000 nm), where higher imaging depth can be achieved without complications from tissue autofluorescence.

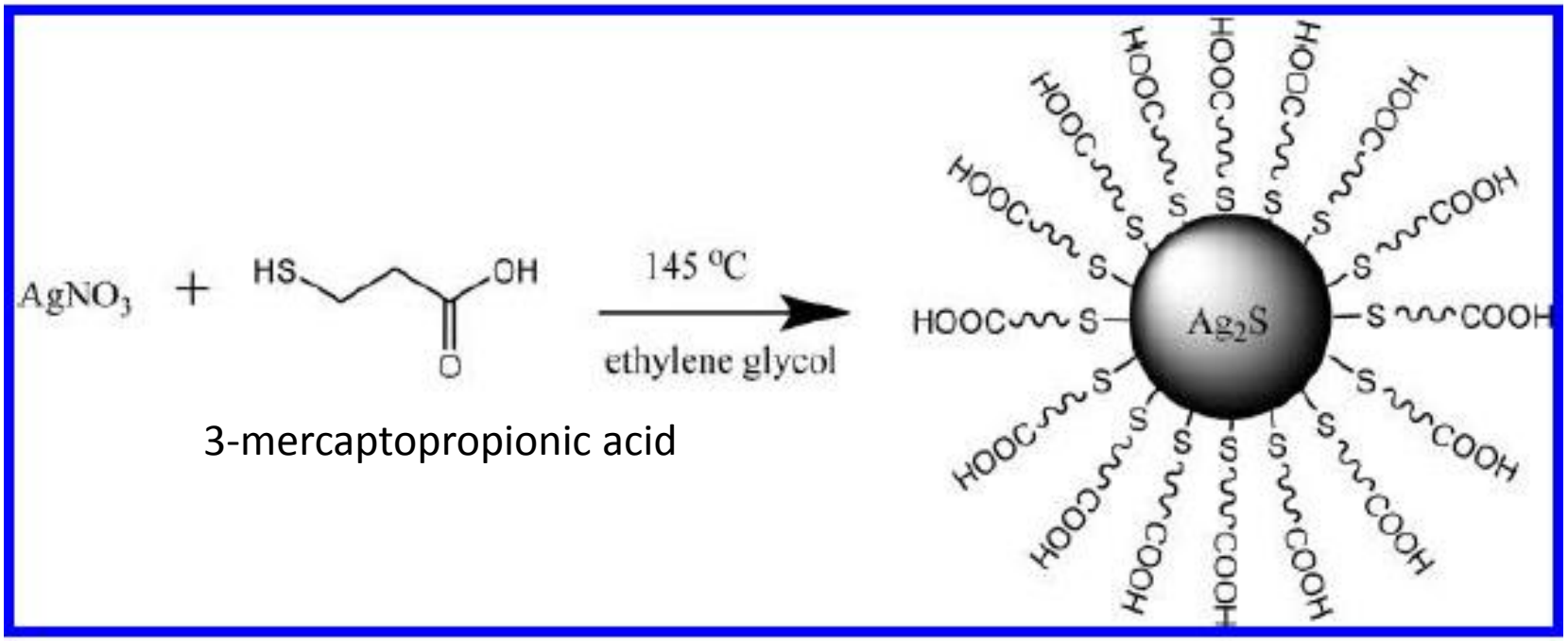
In this study

- Successfully synthesized water-soluble Ag_2S QDs with a wide range emission (from 520 to 1150 nm).

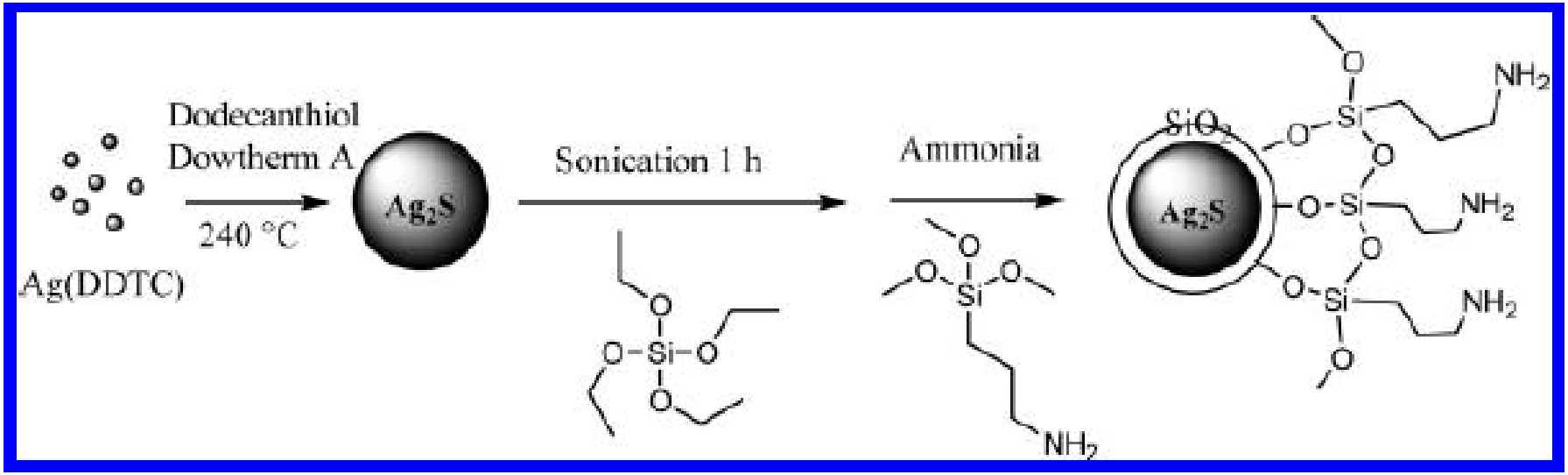
- To deliver the QDs selectively into tumor cells and tissue, they conjugated a cyclic peptide, arginine-glycine-aspartic acid (D)phenylalanine-lysine (cRGDfk) to the QDs for targeting the $\alpha_v\beta_3$ integrin receptor (ABIR),which is up-regulated in tumor cells.

RESULTS AND DISCUSSION

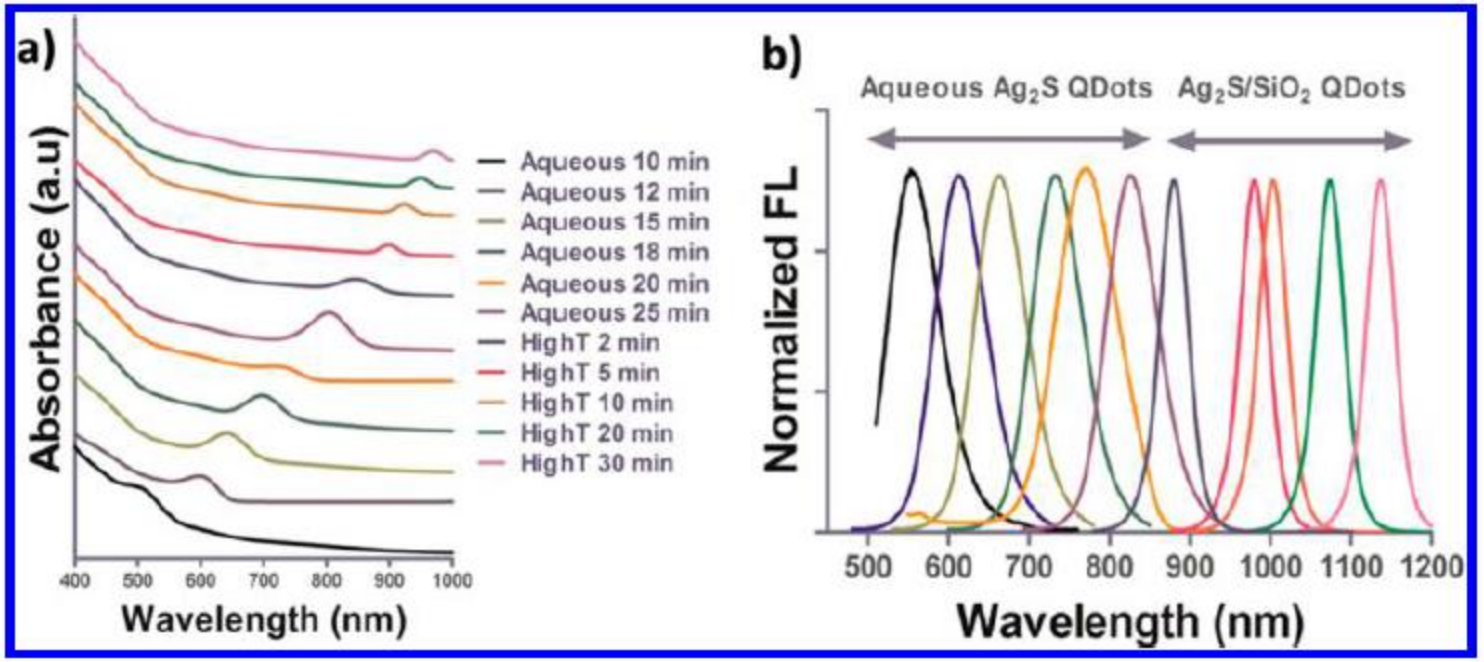
Synthesis of Ag2S QDs in Viscous Media to Produce an Array of Distinct Luminescent Nanoparticles.



Synthetic procedure for constructing water-soluble Ag_2S QDs with emission up to 820 nm.



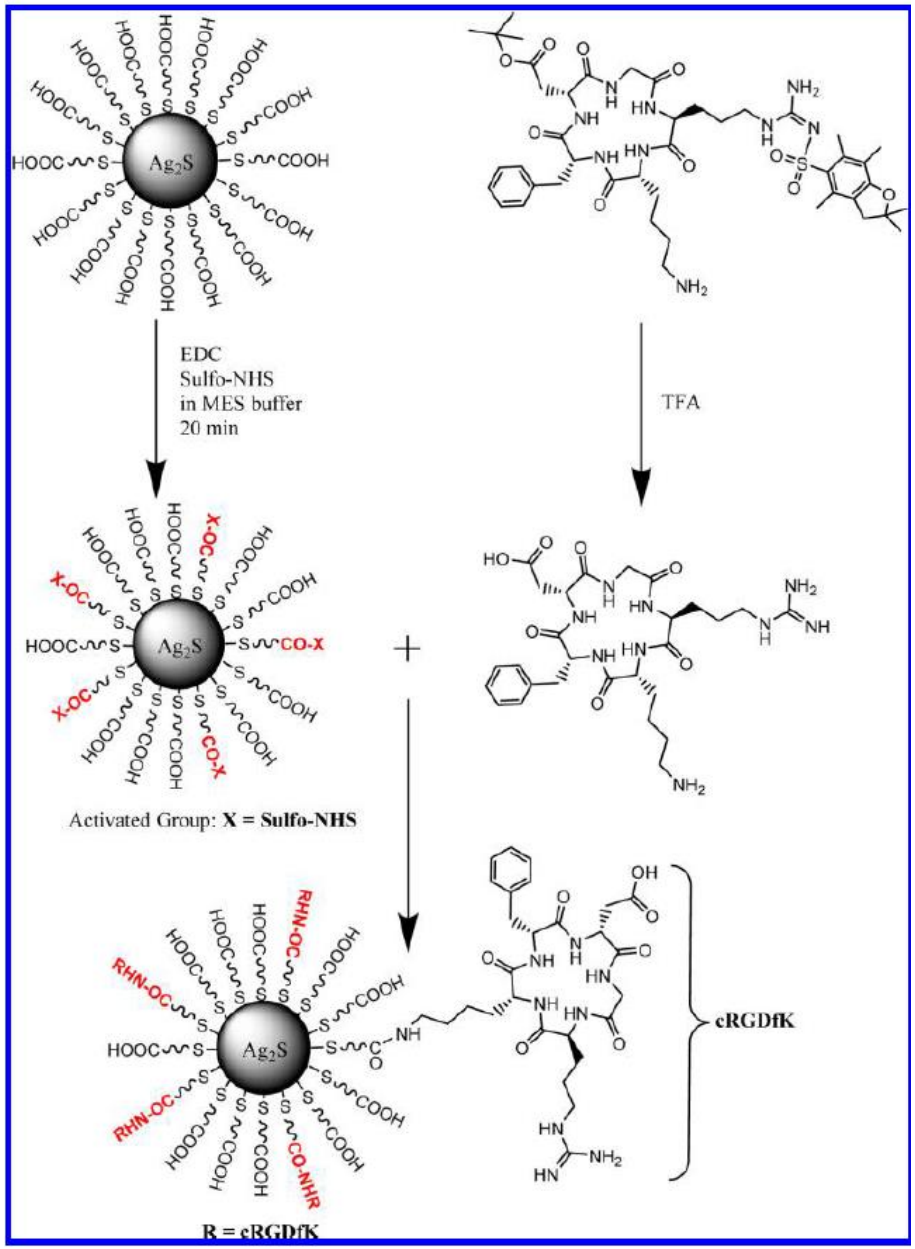
Synthetic procedure for silica-coated Ag_2S QDs with emission wavelengths in the 840 to 1200 nm spectral range.



Temporal evolution of the optical properties of Ag_2S QDs. Absorption (a) and normalized emission spectra (b) of Ag_2S QDs and Ag_2S/SiO2 QDs

QDs	Emission Maximum	Size (nm)	Quantum yield (ϕ)
Aqueous Ag_2S QDs Ex- 488 nm	548 nm	1.48 ± 0.89 (± 60%)	4.4 %
	606 nm	2.33 ± 0.99 (± 42%)	5.1 %
	656 nm	2.97 ± 1.12 (± 37%)	6.6 %
	726 nm	3.89 ± 1.18 (± 30%)	7.0 %
	762 nm	5.01 ± 1.15 (± 23%)	12.2 %
	820 nm	6.12 ± 1.09 (± 17%)	14.1 %
Ag_2S/SiO_2 QDs Ex- 785 nm	877 nm	6.55 ± 1.21 (± 18%)	19.1 %
	976 nm	7.22 ± 1.32 (± 18%)	20.2 %
	1000 nm	7.56 ± 1.37 (± 19%)	18.6 %
	1071 nm	8.25 ± 1.44 (± 17%)	20. 0%
	1135 nm	9.10 ± 1.57 (± 17%)	22.7 %

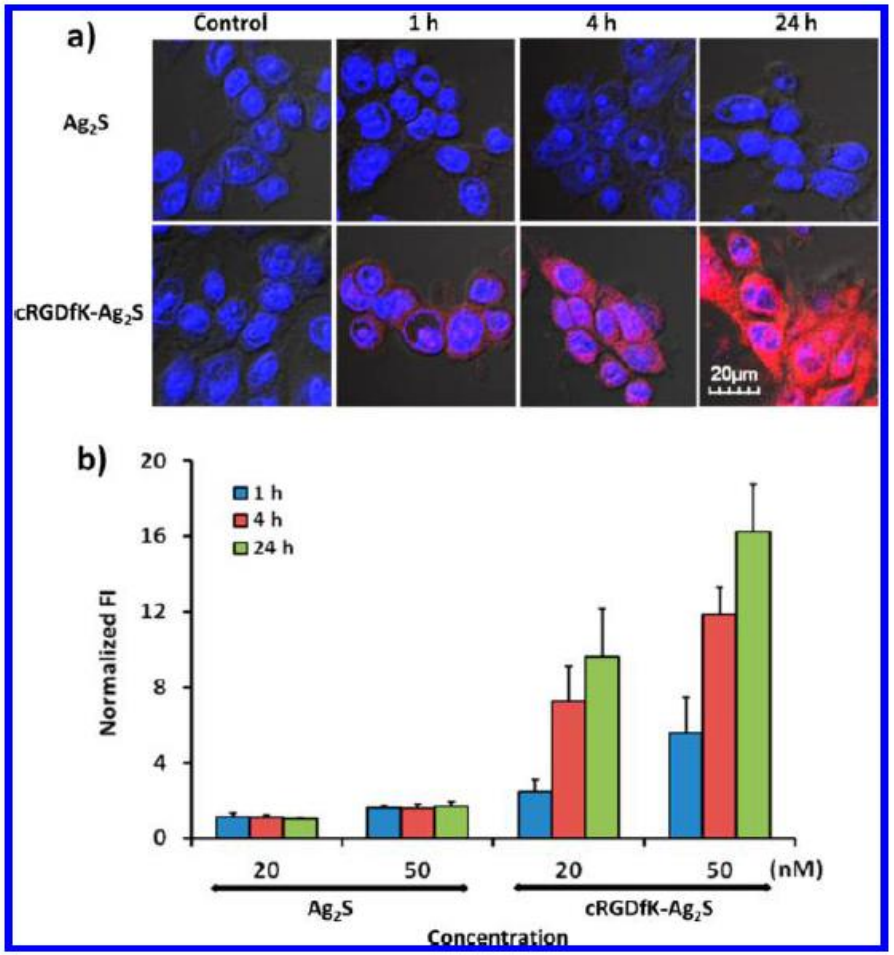
Schematic of the conjugation procedure for cRGDfK-Ag2S QDs.



- The peptide cRGDfK having high affinity towards ABIR was used.

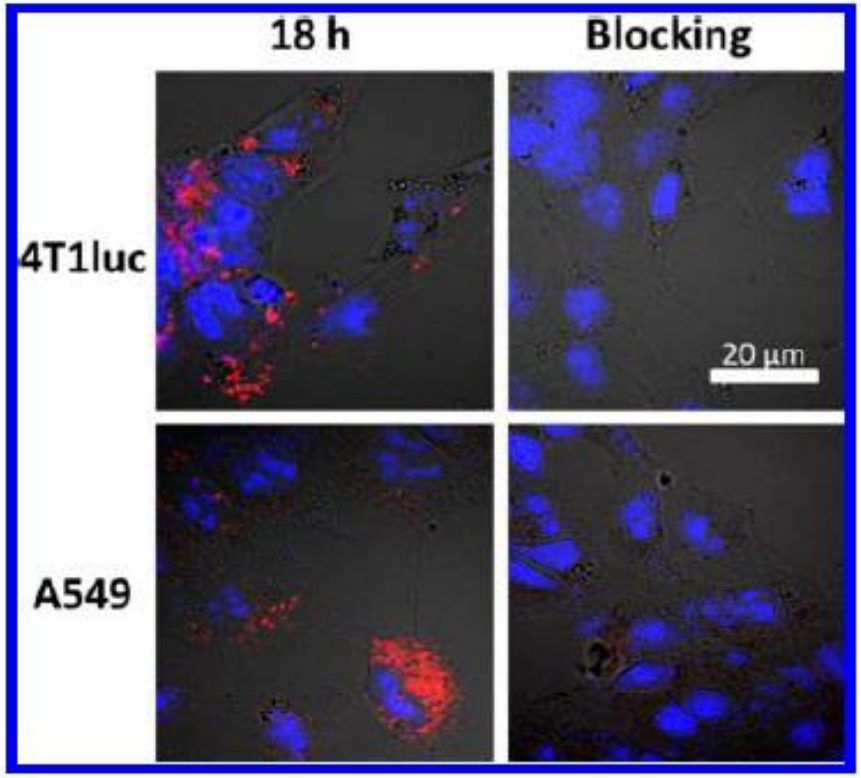
- For NIR fluorescence imaging of cells and deep tissues, 820nmemitting Ag_2S QDs conjugates were used.

ABIR-Mediated Cellular Internalization and Cytotoxicity of cRGDfK-Ag2S QDs- mouse breast cancer cells



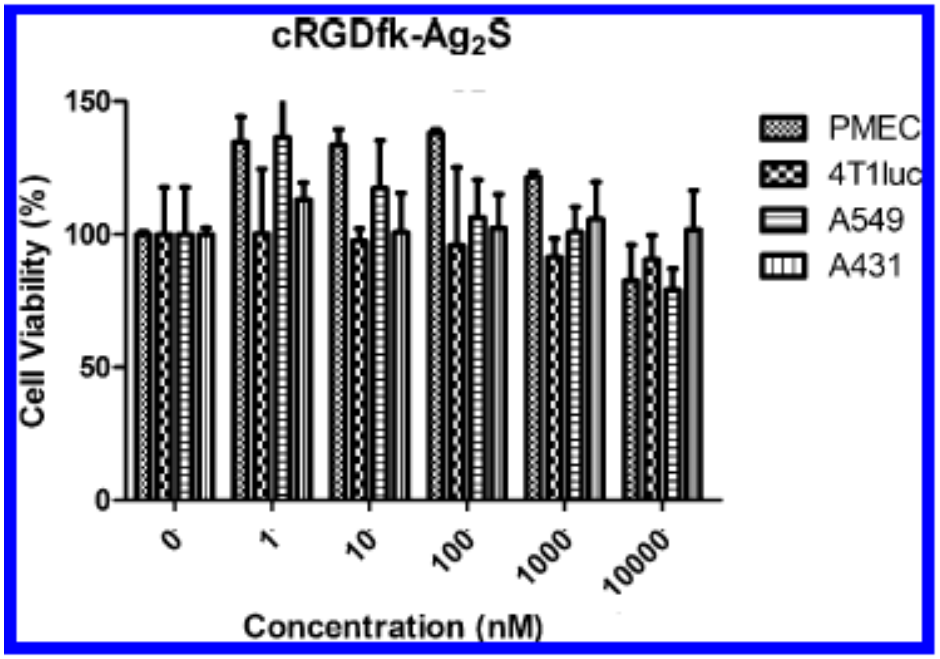
Evaluation of internalization of QDs in 4T1luc cells at different time points: (a) cRGDfK-Ag_2S QDs (red) began to internalize within 1 h and increased up to 24 h of incubation. Fluorescence of cells treated with the control Ag_2SQD remained low (b) Quantitative analysis of the fluorescence intensity (FI) of images in panel a all FI are normalized to background fluorescence

ABIR mediated internalization of cRGDfK QDs



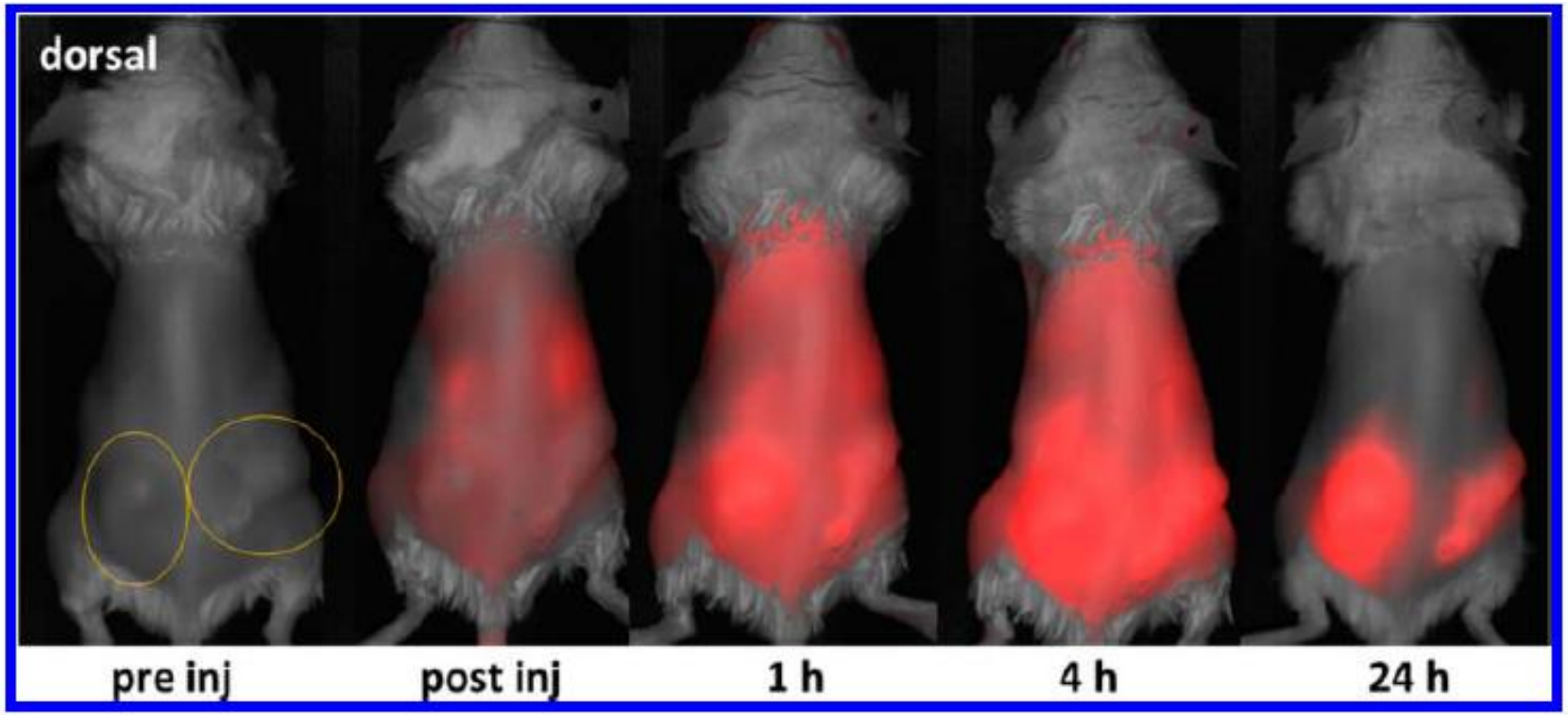
Internalization (left) and inhibition (right) of cRGDfK-Ag2S QDs in 4T1luc and A549 cells using 2 µM cRGDfK peptide and 20 nM cRGDfK-Ag_2S QDs. Red, QD fluorescence; blue, nuclear stain

Cell viability

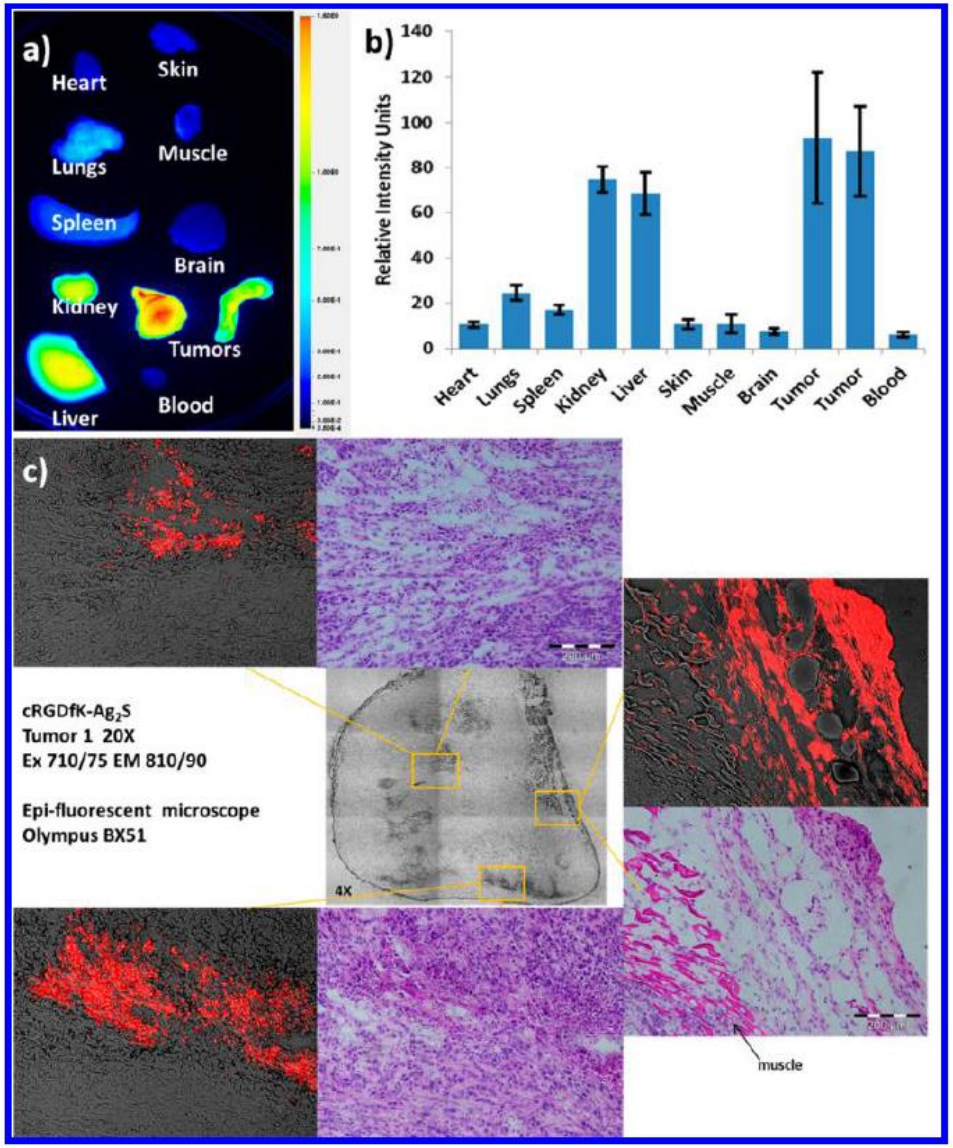


MTT-based cell viability assays of PMEC, 4T1luc, A549, and A431 cells treated with various concentrations of cRGDfK-Ag_2S QDs

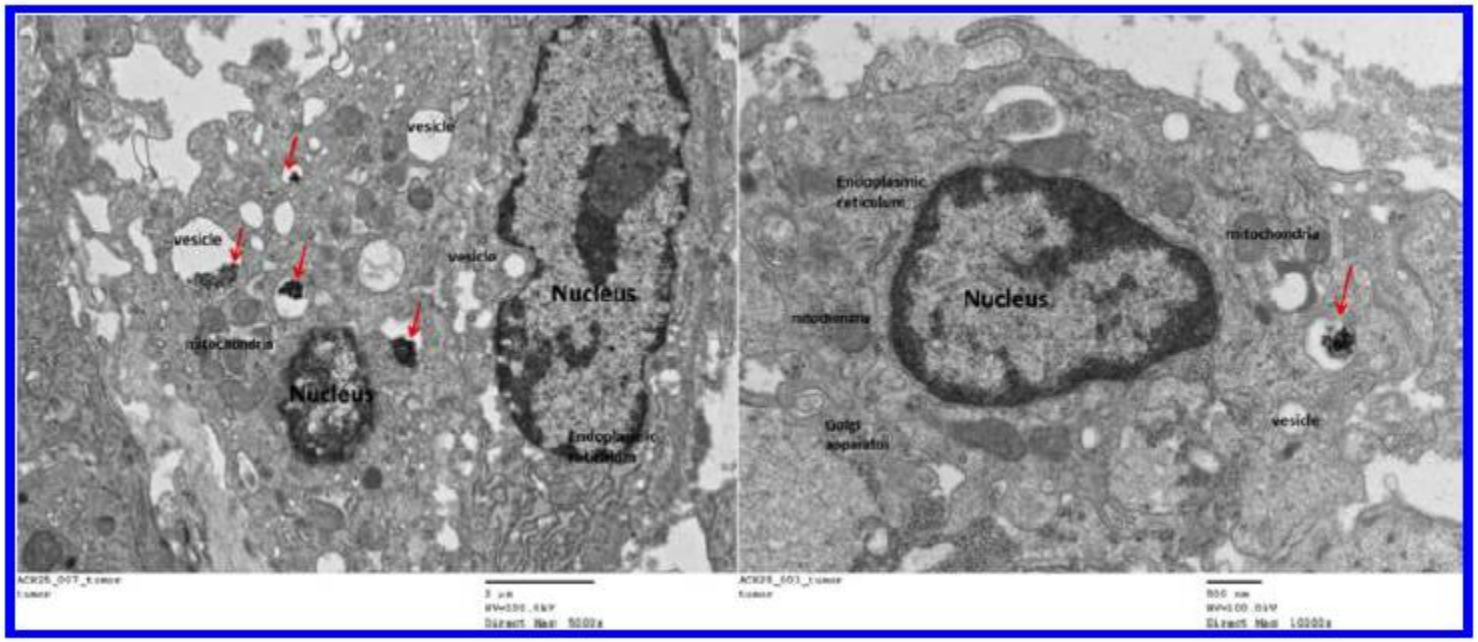
Biodistribution profile of the QDs in rodents



Representative in vivo fluorescence imaging of cRGDfK-Ag_2S in 4T1luc tumor-bearing Balb/c mouse at different time points after intravenous administration. Circles indicate bilateral subcutaneous tumor locations. Red color: QD fluorescence.



(a) ex vivo fluorescence image of organ tissues from 4T1luc bilateral tumor bearing mice. (b) Relative fluorescence intensity analysis of different organs and tissues. (c) NIR fluorescence and hematoxylin and eosin (H&E) stained images of QDs in tumor tissue. Brightfield image is 4cross section of the entire tumor. Boxes indicate areas of the tumor that show NIR fluorescence.



TEM analysis of cRGDfK-Ag_2S QDs internalized into tumor cells. Red arrows indicate cRGDfK-Ag_2SQD accumulation. Scale bar: 2 µm (left) and 0.5 µm (right).

• The QDs were clustered in a subcellular location that appeared to be endocytic vesicles. This suggests that cRGDfK-Ag_2S QD internalization was mediated by endocytosis.

Conclusion

• Developed a modular and simple approach to prepare stable water-soluble NIR Ag_2S QDs covering a wide spectral window from 500 to 1200 nm.

• Using representative Ag_2S QDs, successfully conjugated a tumor-avid peptide, cRGDfK, to the QDs. The combination of a small QD core, coating, and tumortargeting peptide maintained the hydrodynamic diameter at less than 10 nm.

• Both cell and small animal studies demonstrate the high selective uptake in tumor cells and tissue, respectively.

• Unlike most hardcore nanoparticles, higher uptake of cRGDfK-Ag_2S QDs was observed in tumors relative to either the kidneys or liver.

• The simplicity of synthesis, significant renal excretion of unbound QDs, rapid extravasation to tumor tissue, and selective retention in tumors supports the use of this nanoplatform formolecular imaging of diseases and the monitoring of treatment response

Future plan

Understanding the clusters of silver sulfide.

Reaction of this clusters with other Ag, Au clusters or Nps.