

Superparamagnetic Gold Nanoparticles Synthesized on Protein Particle Scaffolds for Cancer Theragnosis

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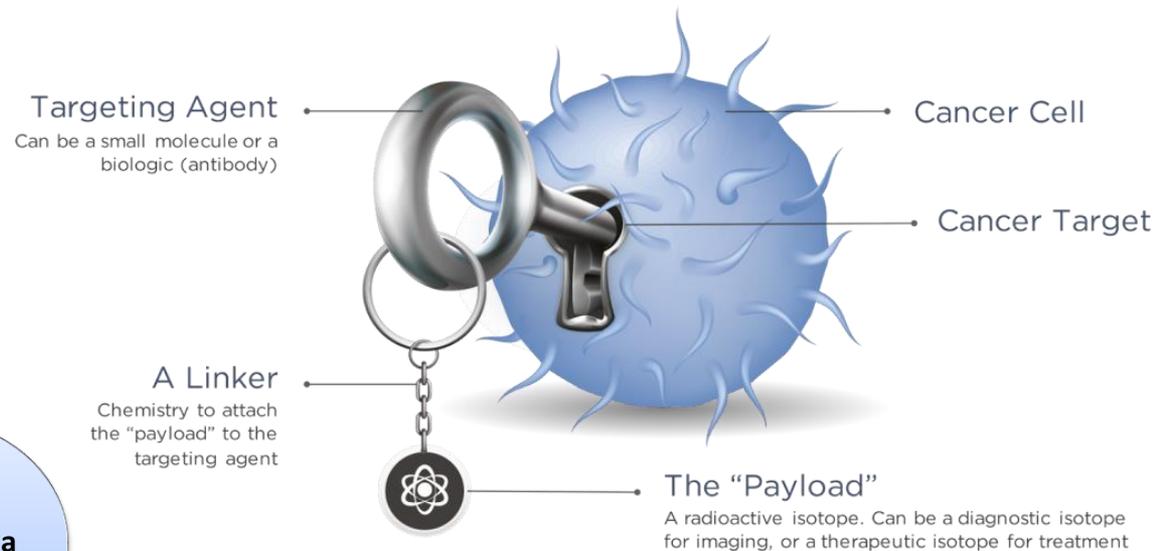
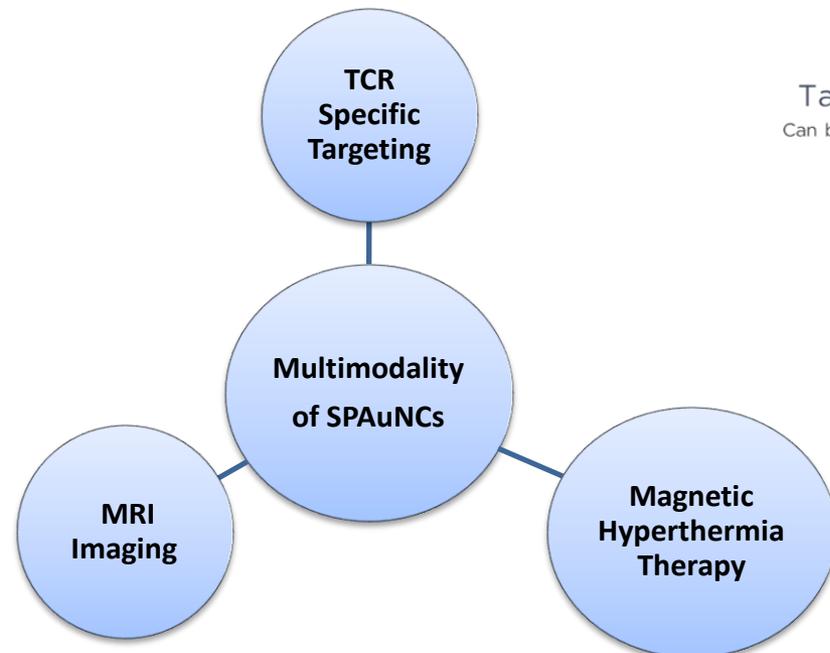
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Introduction

- Theragnosis: Therapy + Diagnosis.
- Superparamagnetic gold-nanoparticle clusters (SPAuNCs) were synthesized on engineered **viral capsid particle** carrier that present **peptide ligands targeting a tumor cell receptor (TCR)**.



<http://www.telixpharma.com>

Significance

Drug Resistance Updates 23 (2015) 69–78



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Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drug



Microenvironment acidity as a major determinant of tumor chemoresistance: Proton pump inhibitors (PPIs) as a novel therapeutic approach



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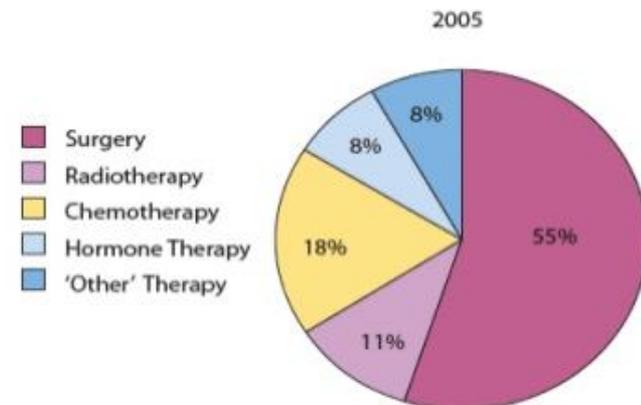
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- Cancer cells have the tendency to develop **Chemoresistance** to the Cancer drugs.
- Thus, there is a need to develop various **alternative modes** of treatments.
- Targeting the cancer cell is a major challenge as **the cancer biomarkers vary** from patient to patient and tumor to tumor within a patient.



<https://www.cancer.gov/about-cancer/treatment/types>

Ultrasmall CuCo_2S_4 Nanocrystals: All-in-One Theragnosis Nanoplatfom with Magnetic Resonance/Near-Infrared Imaging for Efficiently Photothermal Therapy of Tumors

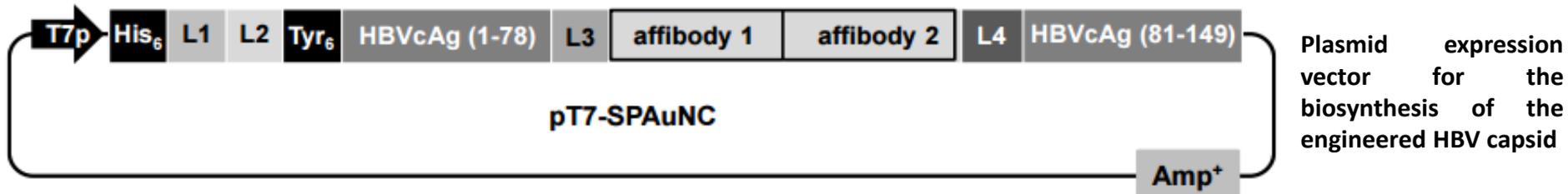
Bo Li, Fukang Yuan, Guanjie He, Xiaoyu Han, Xin Wang, Jinbao Qin, Zheng Xiao Guo, Xinwu Lu, Qian Wang, Ivan P. Parkin, and Chengtie Wu**

- Ultrasmall copper-based ternary chalcogenide nanoparticles, CuCo_2S_4 Nanocrystals were prepared by a one-pot hydrothermal route.
- Low toxicity and bioavailability.
- MR resonance imaging is necessary to overcome the challenges in photothermal imaging.
- They need to be further conjugated **tumor targeting agents**.

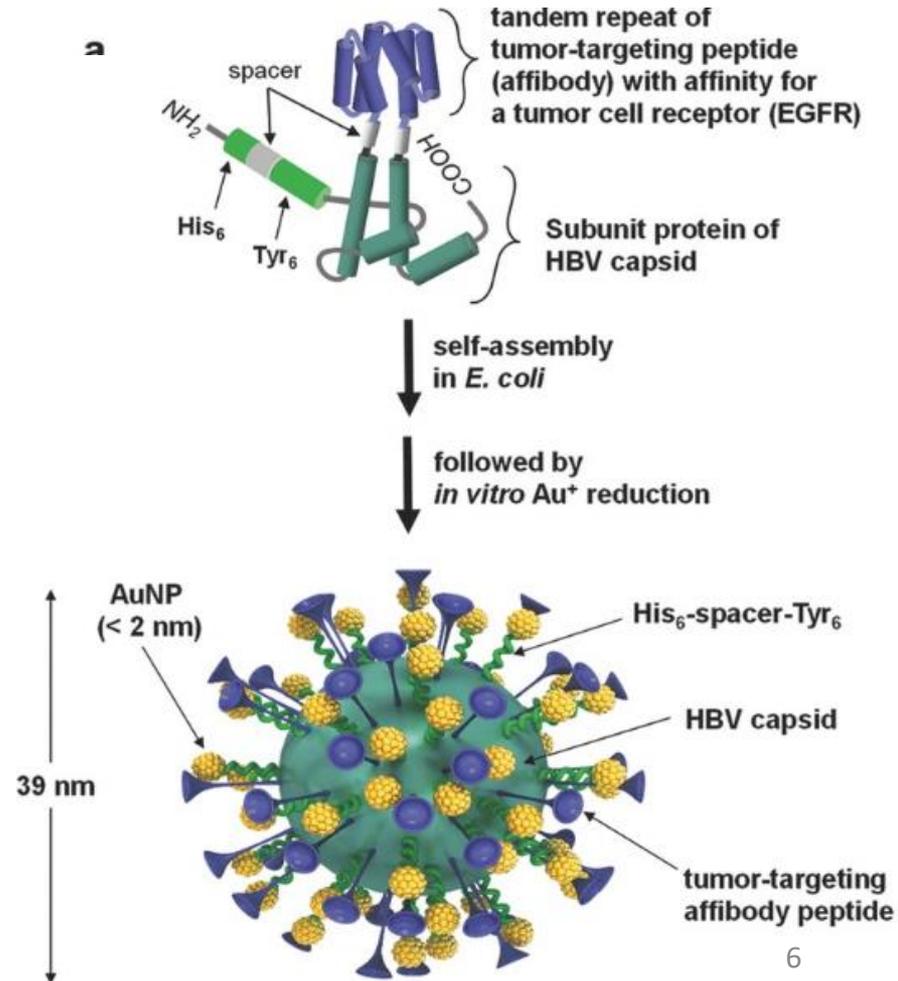
Relevance

- Synthesis of Nanoparticle on **target specific biomolecular scaffolds** to facilitate in vivo imaging.
- **Magnetic and Surface Plasmon resonance properties** can be changed through interaction with **amino acids**.

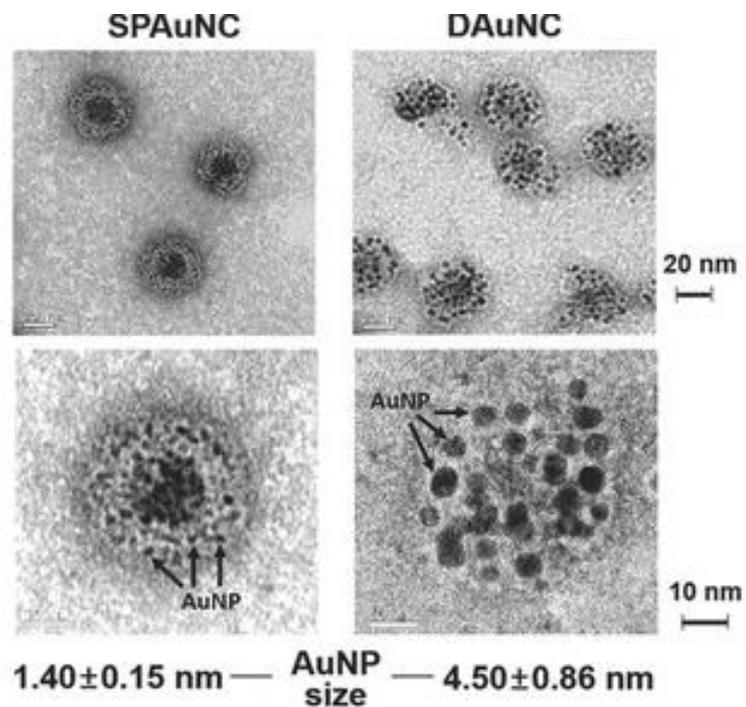
Synthesis of SPAuNCs



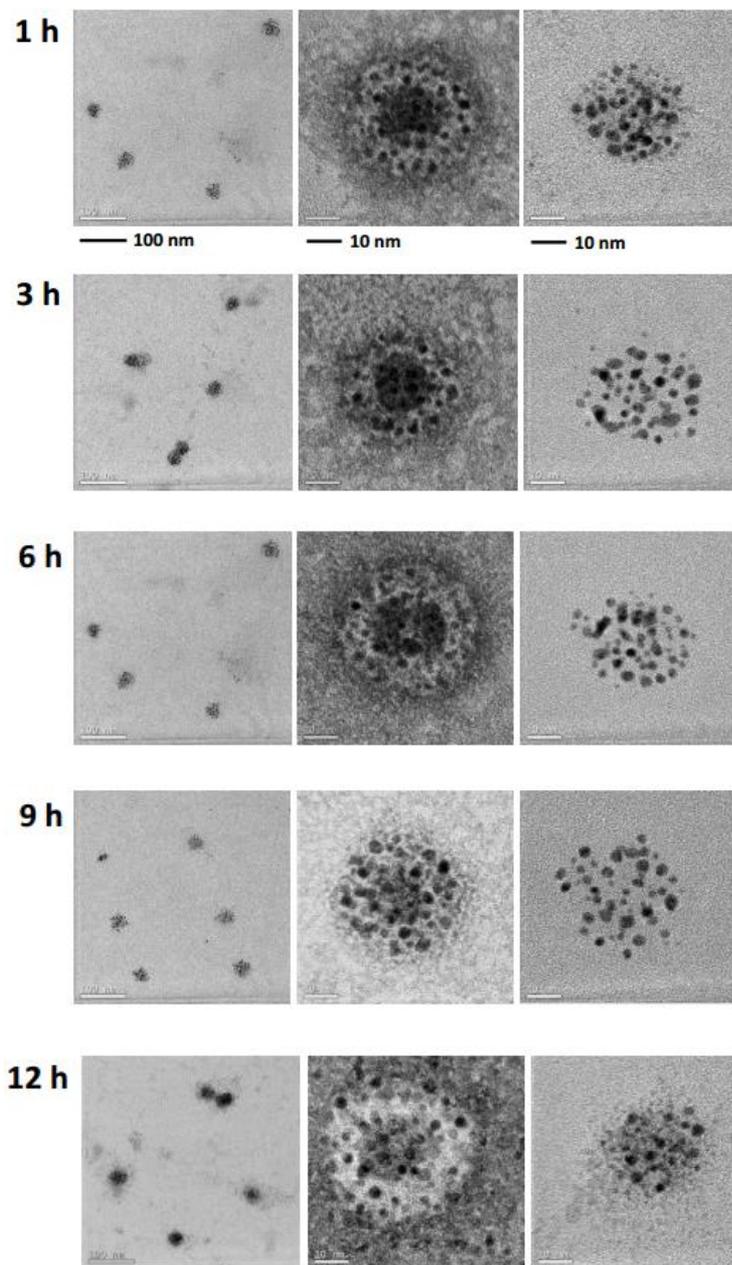
- Genetic modification to express a **HBV core protein**.
- The protein has a tandem repeat of **affibody peptide**: strong affinity for human epidermal growth factor (EGFR) which are overexpressed in various tumours (Adenocarcinoma, Epidermal carcinoma etc).
- The modified HBV core protein was **expressed and self assembled in *E.coli***.
- The engineered HBV capsids were mixed with chloro(trimethyl phosphine) gold(I) to chemisorb the gold ions $[(CH_3)_3PAu^+]$ through the **coordination bond** with the **surface-localized His₆**.
- The chemisorbed gold ions were **reduced** by $NaBH_4$ to form cluster of AuNPs on the Capsid.



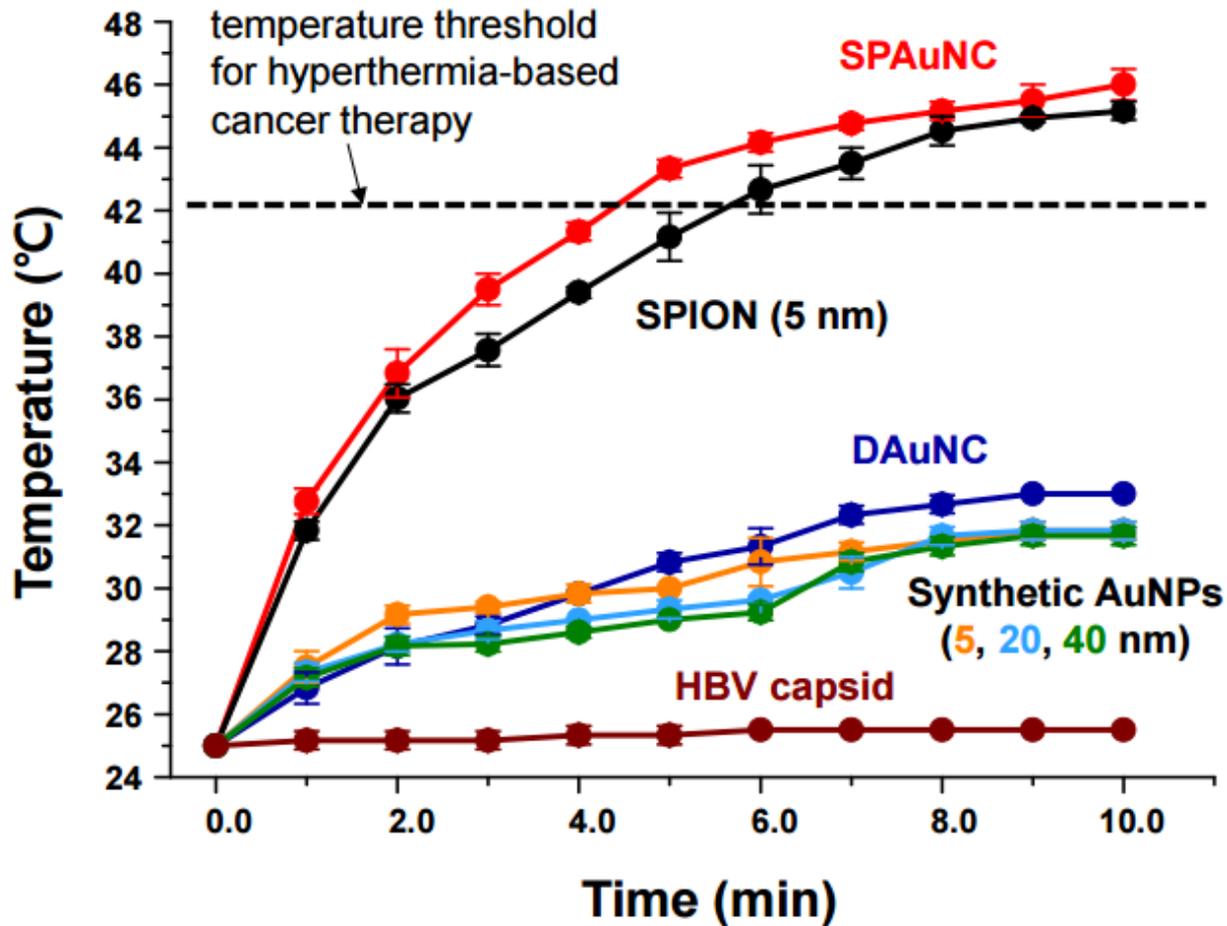
Results and discussion



TEM analysis of SPAuNCs (38.7nm) and DAuNCs (41.6nm)

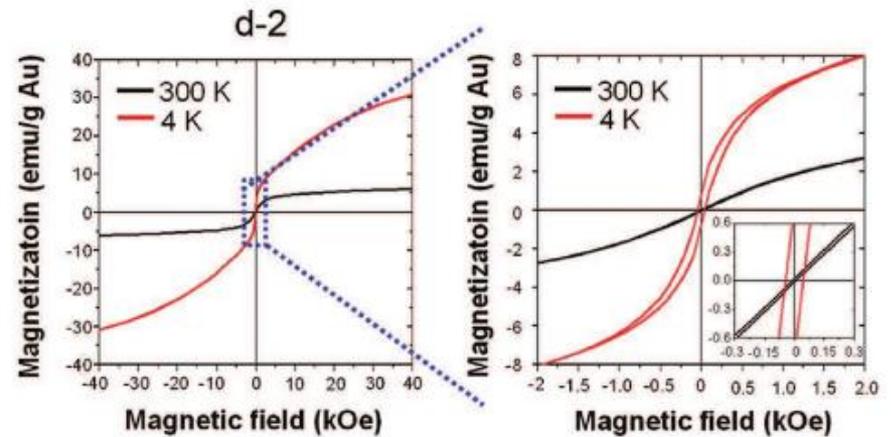
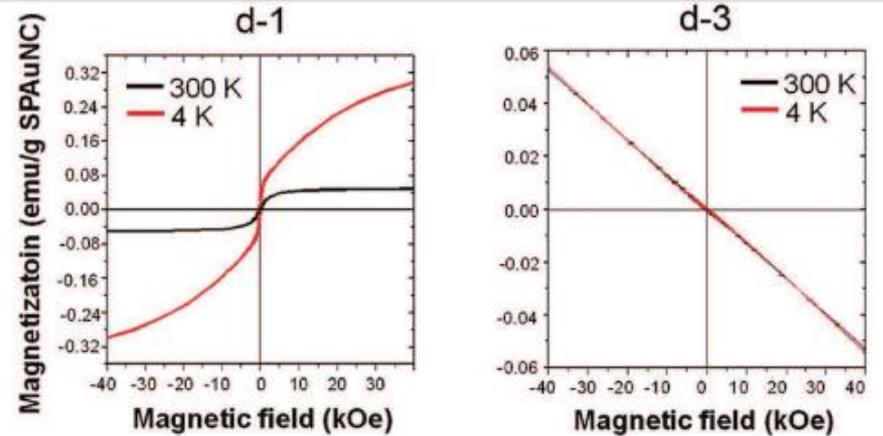
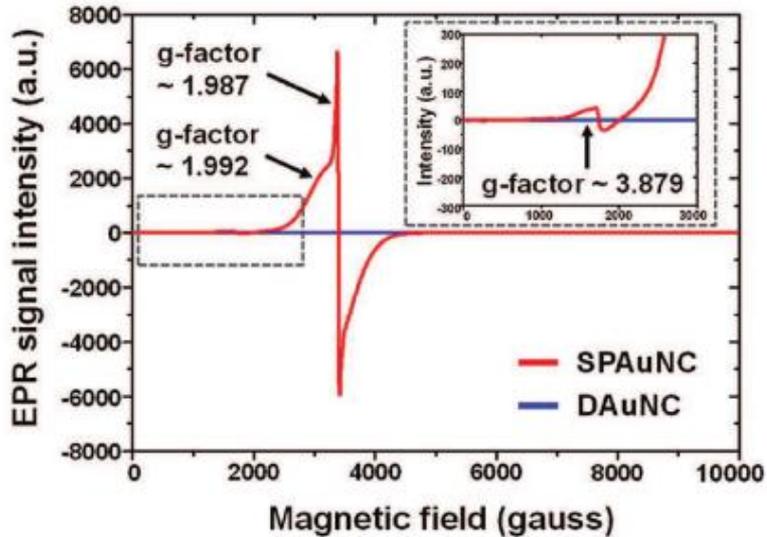


Time-course TEM images of SPAuNC in 50% human serum solution at 37.5 °C to test stability



Magnetic hyperthermia effect, estimated through the time-course measurement of temperature increase in the PBS solutions containing SPAuNC, SPIONs (Superparamagnetic Iron oxide Nanoparticles) (5 nm), DAuNC (Diamagnetic AuNC), synthetic AuNPs (5, 20, 40 nm), and engineered HBV capsid under an AMF (10 kW/360kHz).

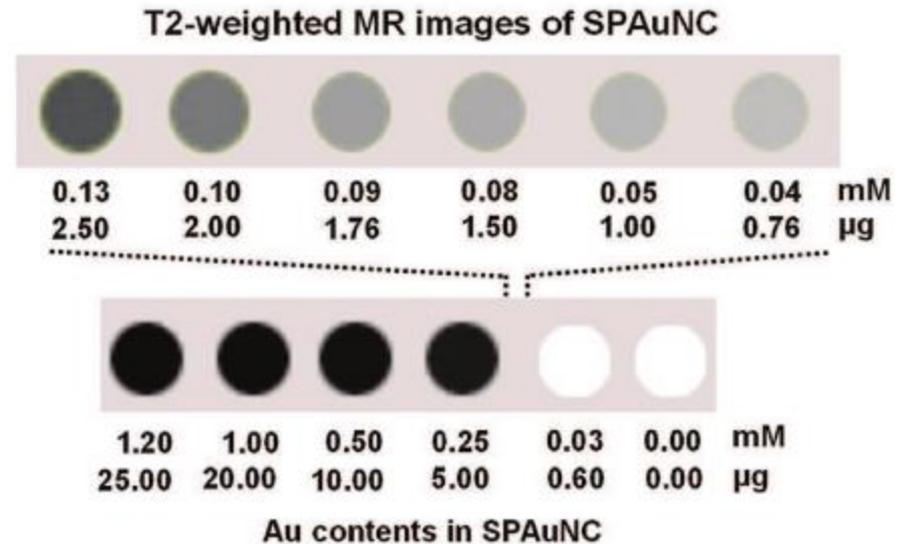
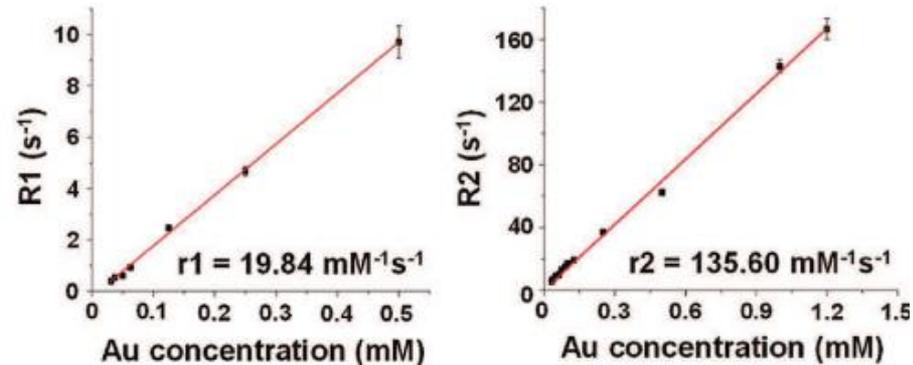
Magnetic Properties



EPR spectra of SPAuNCs and DAuNCs with g-factors (dimensionless magnetic moment)
Features at 1700, 3200 and 2000 to 4500 G correspond to that of Superparamagnetic Iron oxide Nanoparticles

Results of SQUID magnetometer analysis of SPAuNC/AuNPs (d-1, d-2) and DAuNC (d-3)

MRI contrast

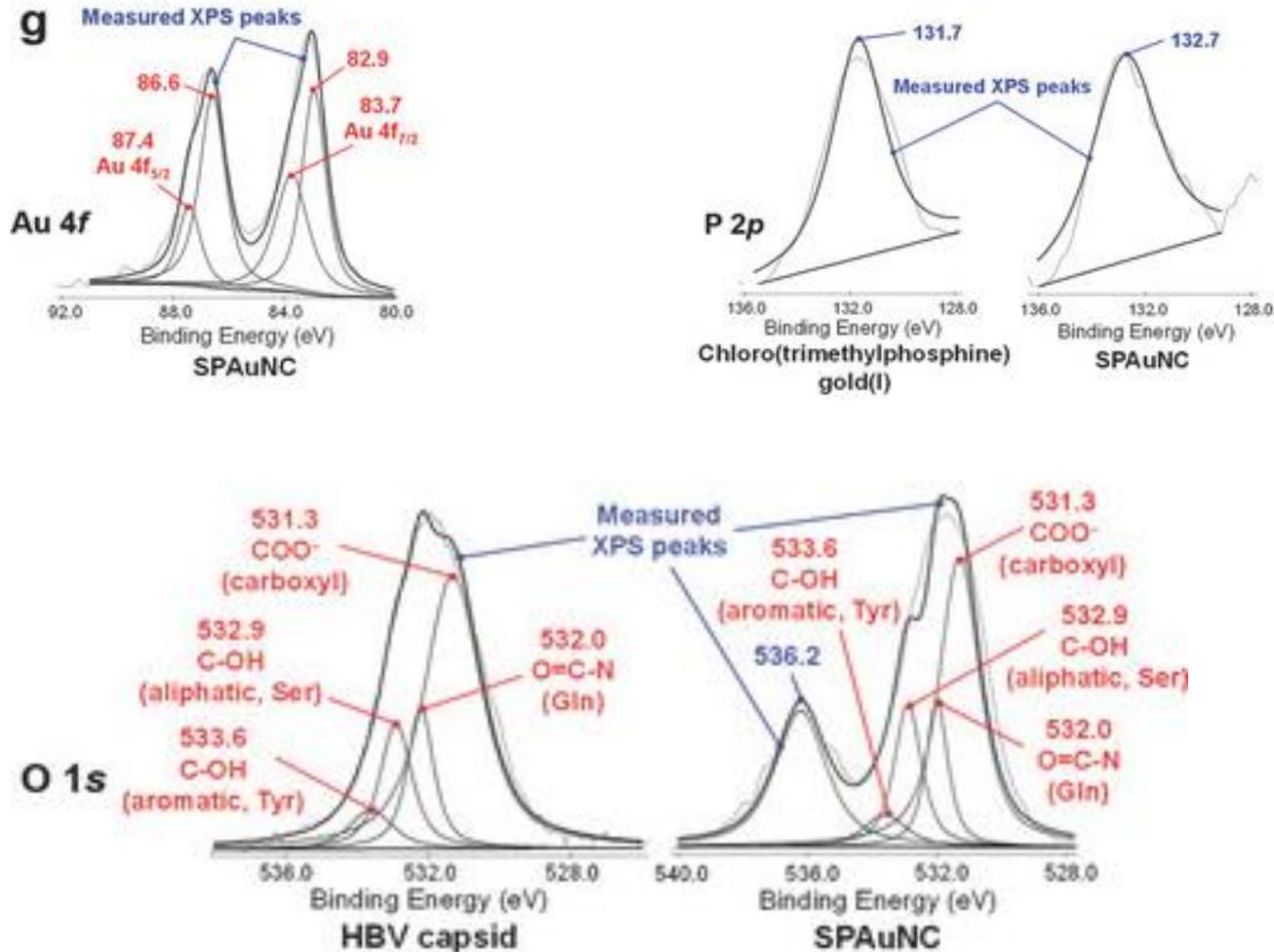


T_1 and T_2 relaxation rates (R_1 , R_2) and relaxivities (r_1 , r_2) of SPAuNC in PBS.

Higher the ratio of r_2 to r_1 is, the more the contrast agent is suitable for T_2 -weighted imaging. The relaxivity ratio (r_2/r_1) of SPAuNC (aff +) is 6.8 which is comparable to that of commercial T_2 -contrast standard, Resovist (i.e., 6.2)

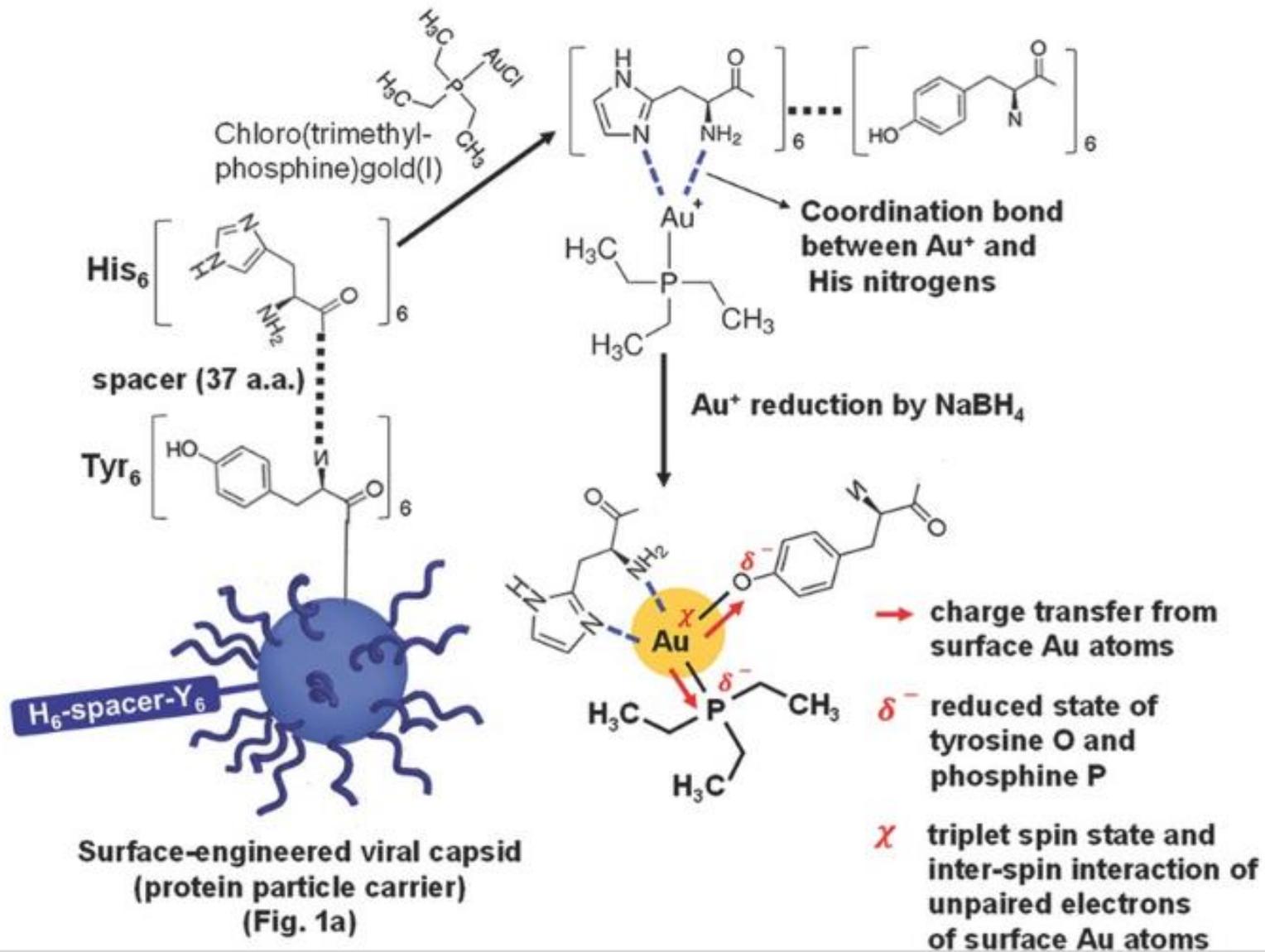
T_2 -weighted MR images of SPAuNC at various Au contents.

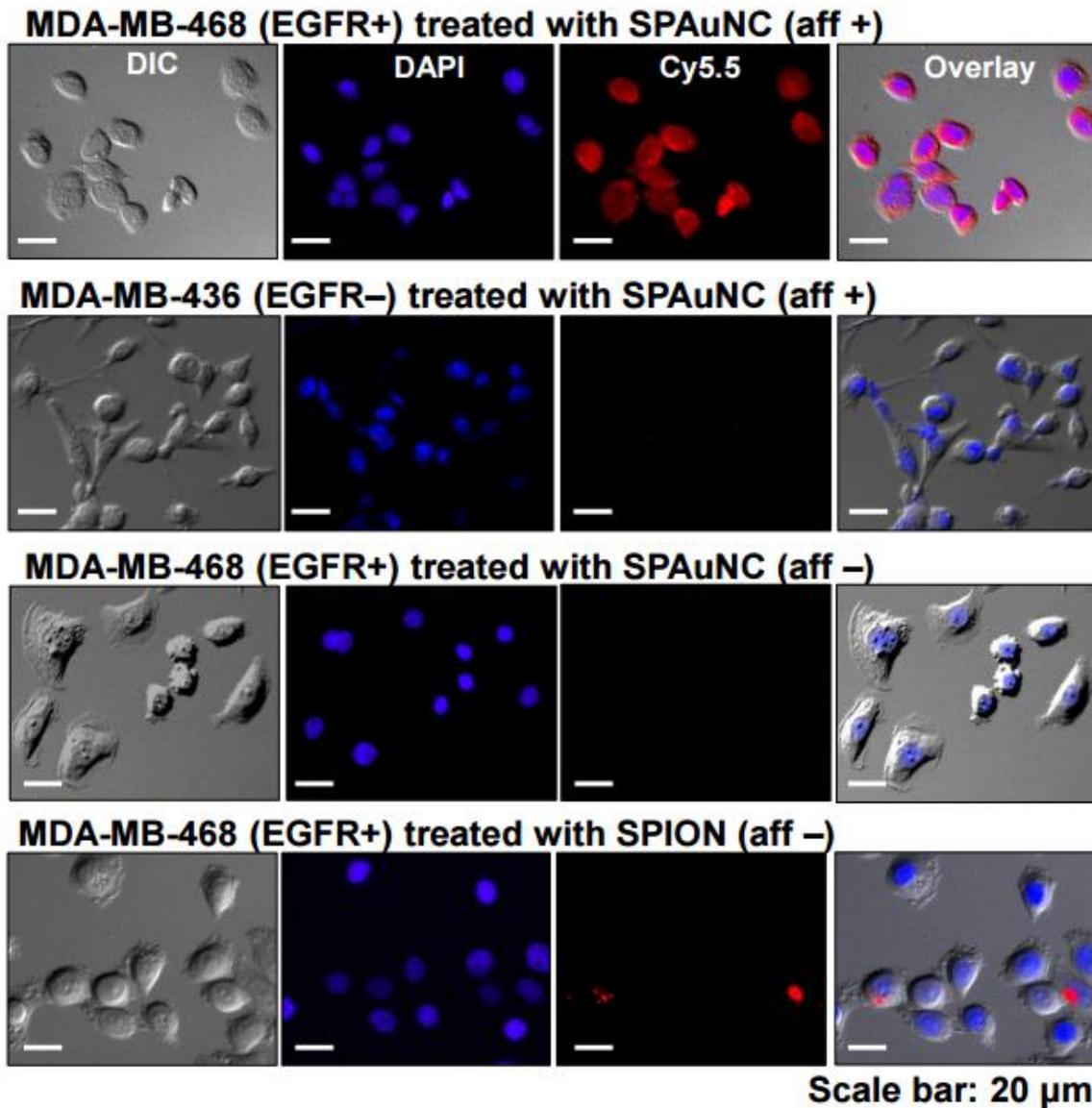
XPS Studies



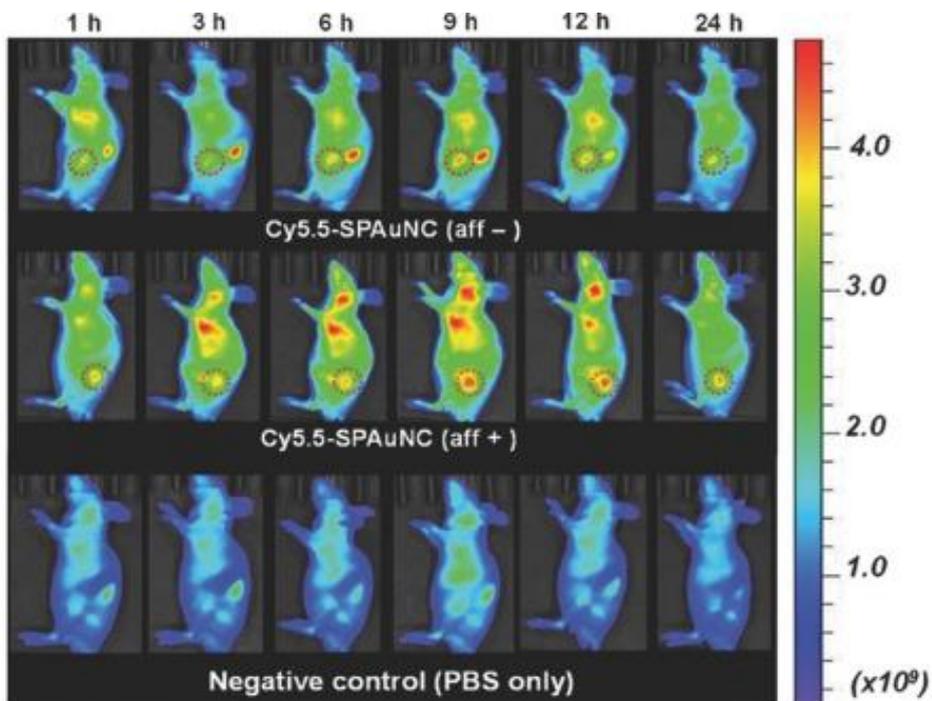
Gold-chemisorbed oxygen is the side chain oxygen of Tyr₆, because the Tyr₆ is located near His₆ that is combined with (CH₃)₃PAu₊ via a coordination bond

Induction of Magnetism



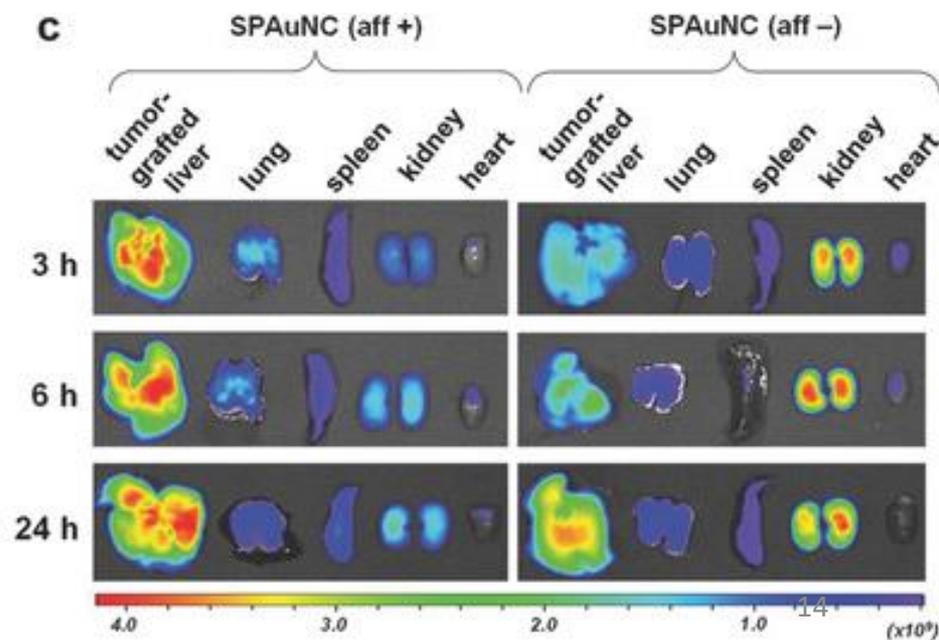


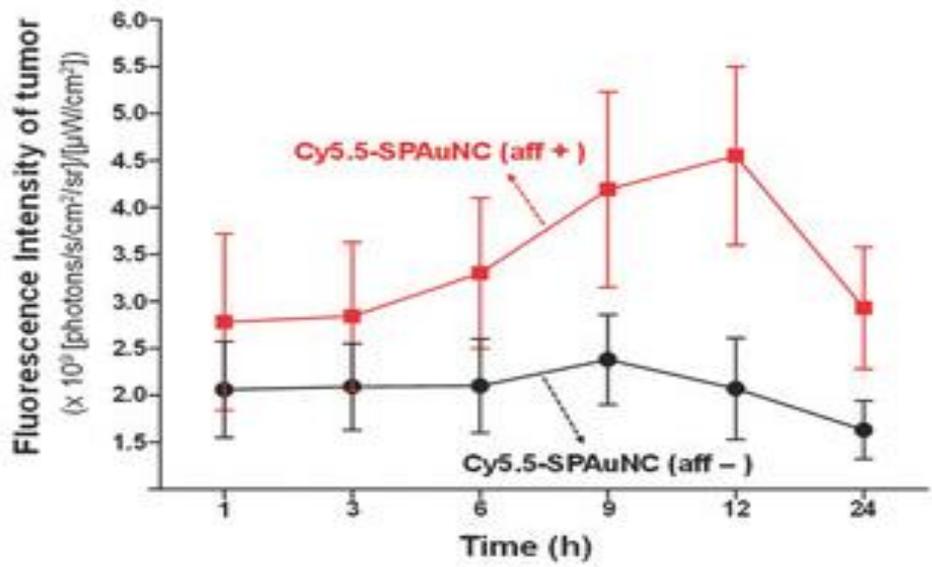
Fluorescence images of in vitro cultures of EGFR-expressing (MDA-MB-468) and 4 EGFR-free tumor (MDA-MB-436) cells treated with SPAuNC (aff +), SPAuNC (aff -), 5 or SPION that were all labeled by Cy5.5.



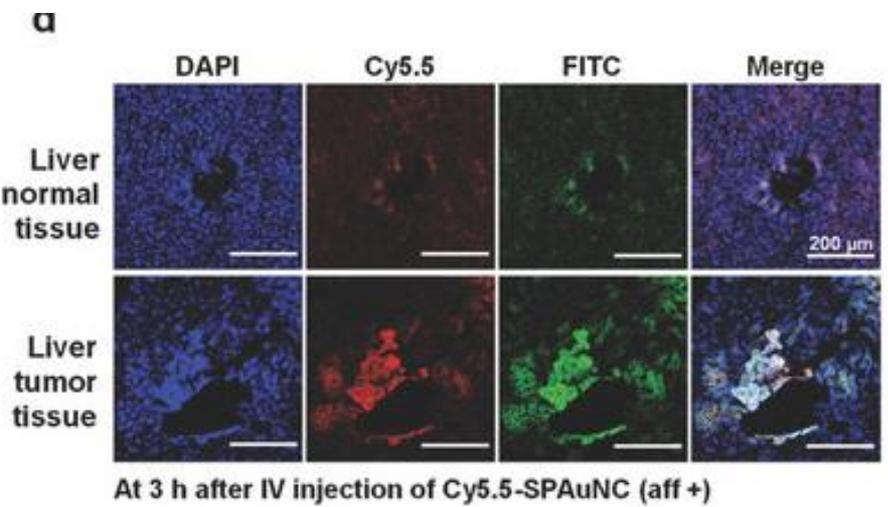
Ex vivo NIRF images of tumor (MDA-MB-468)-grafted liver and four major organs (lung, spleen, kidney, and heart) at the predetermined time points for 24 h after the intravenous injection of Cy5.5-SPAuNC (aff + or aff -) to the liver-tumor-bearing mice.

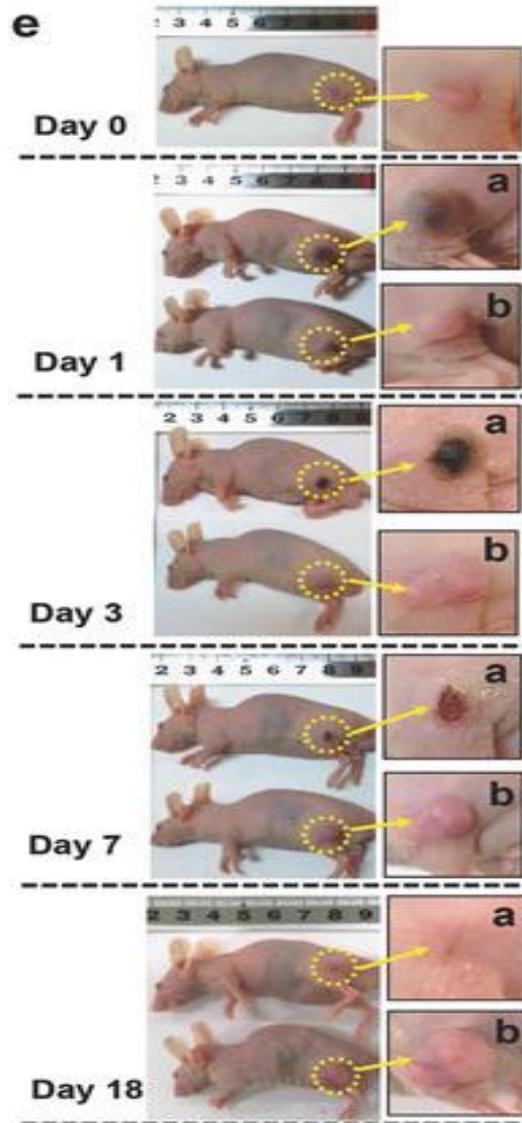
NIRF images of mice bearing a subcutaneous tumor (MDA-MB-468) at the predetermined time points for 24 h after the intravenous injection of Cy5.5-SPAuNC (aff + or aff -). (The dotted circles indicate the tumor.)





Time-course variation of the NIRF intensity from the MDA-MB-468 tumor of the mice

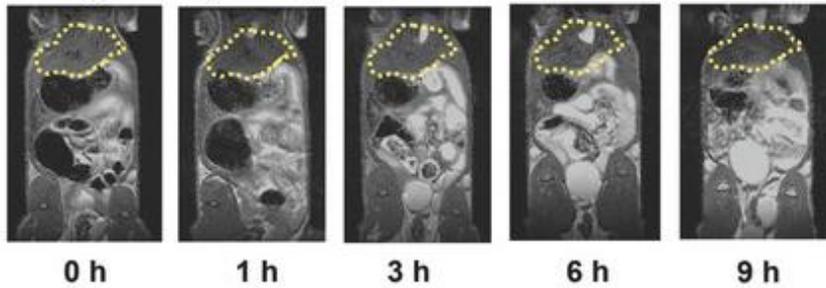




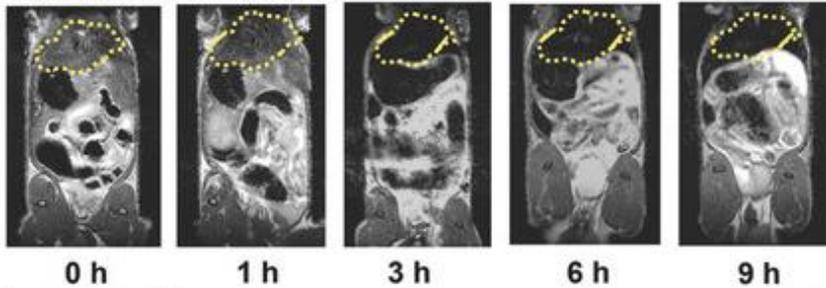
Time-course photographic images of the mice bearing a subcutaneous tumor (MDA-MB-468) (n = 6 with the survival rate of 100%) that were treated (case a) or not treated (case b) by SPAuNC (aff +). (At 9th h after the intravenous injection of SPAuNC (aff +) to the subcutaneous tumorbearing mice, the AMF (360 kHz, 10 kW) was applied for 15 min using a radio frequency generator.)

SPAuNC + AMF AMF only SPAuNC + AMF AMF only SPAuNC + AMF AMF only

Healthy mouse (control)



Liver tumor-bearing mouse



Pre-injection

After IV injection

Time-course T2-weighted MR images of the mice bearing a liver tumor (MDA-MB-468) and healthy mice using SPAuNC (aff +) as a contrast agent. (The yellow dotted circles indicate liver.)

Summary

- **In vivo denaturation** of the subunit proteins is accelerated under the tumor-killing hyperthermia condition.
- This leads to the spontaneous disassembly of the viral capsid, followed by the **release of the individual small AuNPs**.
- The released small AuNPs easily pass through **glomerular filtration** and are effectively removed through renal excretion.
- The SPAuNCs exhibited a notable **multimodal performance** of both **subcutaneous** and **deep-tissue tumors** in live mice.
- The SPAuNCs showed excellent **biocompatibility** without **in vivo accumulation problems**.

Thank you