Design of Biocompatible Nanoparticles for Probing Living Cellular Functions and Their Potential Environmental Impacts

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Scientific Problems & Challenges

Advances in nanoscience and nanotechnology promise a wide variety of potential applications of nanoparticles (NPs), including nanosensors and nanophotonic probes for molecular imaging, diagnosis and smart drug delivery. However, fundamentals of effects of nanomaterials on living organisms and human health remain not yet fully understood. Addressing this crucial question effectively would enable rational design of eco-friendly materials and regulatory guidelines for safe handling and manufacturing of nanomaterials and making our eco-system and environment healthier and more sustainable.

The primary questions and challenges are: (i) whether the effects of nanomaterials on living organisms are unique or just like other conventional chemicals. (ii) How can one effectively characterize their biological effects and understand their underlying molecular mechanisms, in order to predict and rationally design biocompatible and eco-friendly nanomaterials? Addressing these primary questions are vital to achieve the manufacture safety, to eliminate their potential adverse impact on environments and to ensure healthy and sustainable eco-system and environment.

Results and Discussion

We have synthesized and characterized a mini library of stable and purified noble metal NPs (e.g., Ag and Au NPs) with controlled sizes, shapes and surface properties. We have developed single nanoparticle optical microscopy and spectroscopy to characterize the sizes, shapes and optical properties of single NPs using their localized surface plasmon resonance (LSPR) spectra, and developed single nanoparticle optical rulers to measure the size and shape of single plasmonic NPs *in situ* in real time. We have shown that single noble metal NPs resist photodecomposition and directly imaged single NPs in embryos *in vivo* over time.

We have demonstrated that the early developing zebrafish embryos can serve as an *in vivo* assay to study the biocompatibility and toxicity of nanomaterials. We have systematically studied the size, dose, chemical, and development stage dependent effects of NPs upon embryonic development.

We have used biocompatible photostable single NP optical probes, single-molecule NP optical biosensors (SMNOBS), and photostable optical nanoscopy (PHOTON) to quantitatively image single ligand-receptor interaction on single live cells in real time with both spatial and temporal resolutions[1-11] We have also designed drug nanocarriers to probe multidrug membrane transporters and explored effective drug delivery. We are currently developing these new imaging technologies for capturing functions of individual neurotransmitters at single synapses with single-molecule sensitivity for a wide variety of applications.[12-14]

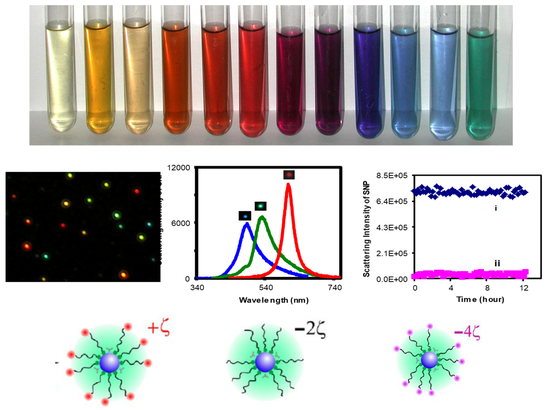

Fig 1: Design and synthesis of a mini-library of NPs, LSPR spectra and images of single NPs, and characterization of photostability of single NPs.

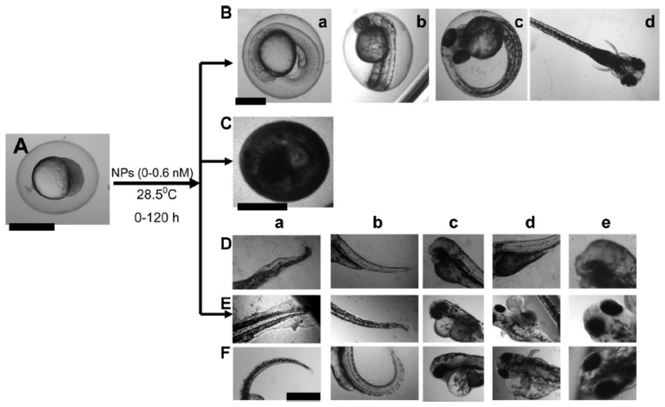

Fig. 2: Early-developing embryos as in vivo assays to study biocompatibility of toxicity of NPs. Scale bars = 500 µm.

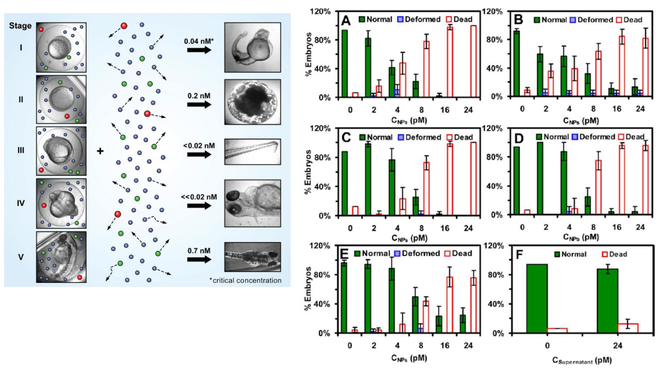

Fig. 3: Study of dose- and stage-dependent effects of the Ag NPs on embryonic developments. Histograms show the percentages of stage I−V embryos, and they have been incubated with (A−E) Ag NPs or (F) the supernatant for 2 h that develop to normal and deformed zebrafish or die in egg water over 120 hpf.

Single-molecule Nanoparticle Optical Biosensors (SMNOBS)

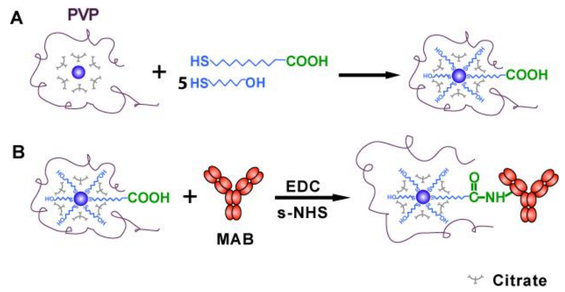

Fig 5: Synthesis of photostable SMNOBS for sensing single TNF-α molecules.

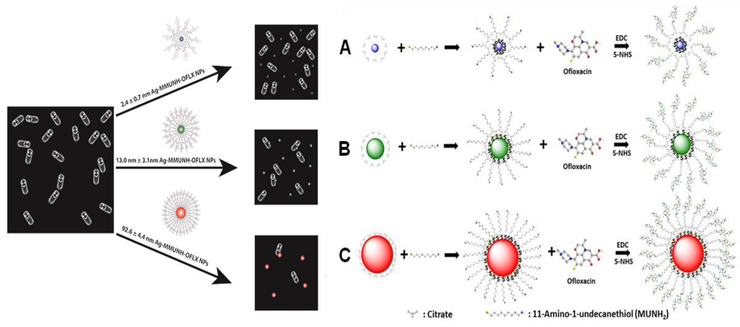

Fig 8: Design of drug nanocarriers for probing multidrug membrane transporter.

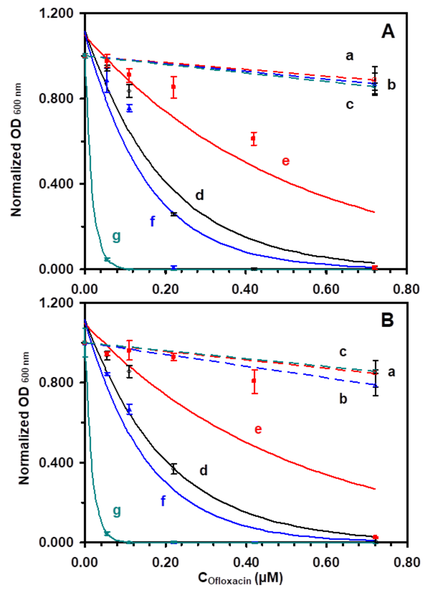

Fig. 9: Dose and size dependent Minimal inhibitory concentration (MICs) of antibiotic nanocarriers against (A) WT and (B) ΔBmrA cells. Plots of normalized $OD_{600\ nm}$ of the cells cultured for 17 h in the modified LB-medium containing (a-c) $AgMUNH_2$ NPs (absence of Oflx, control), (d) Oflx alone, and (e-g) Oflx conjugated with the sized Ag NPs of (e) 2.4 ± 0.7, (f) 13.0 ± 3.1 and (g) 92.6 ± 4.4 nm in diameter, respectively.

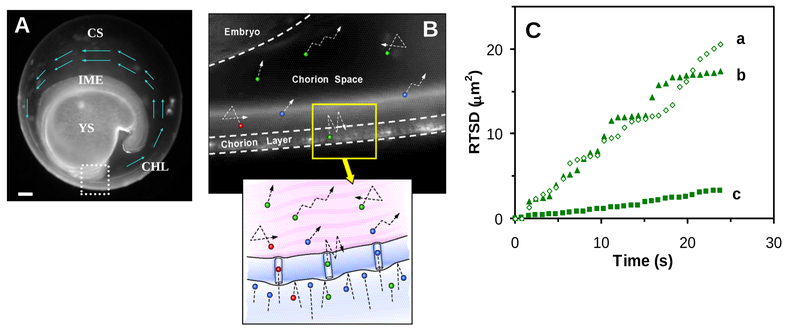

Fig 4: Real-time imaging and probing of nano-environments of a segmentation stage (21 hpf) zebrafish live embryo. Scale bar = 100 µm.

PHOTON (Photostable Optical Nanoscopy) for nm-Resolution Imaging of Single Live Cells

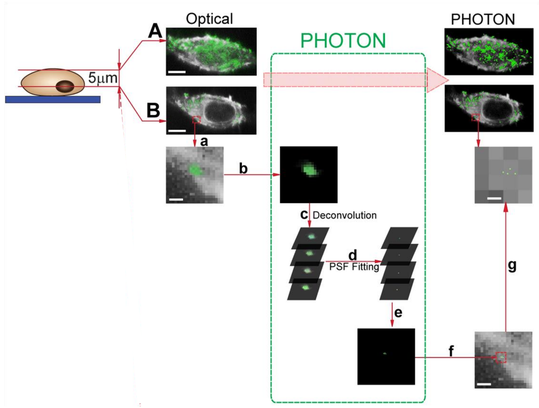

Fig 6: Design of PHOTON for real-time super-resolution single-molecule imaging of dynamics and mechanisms of apoptotic signaling pathways of single live cells: Optical imaging of: single SMNOBS bind with its receptors **(A)** on and **(B)** in single live cells.

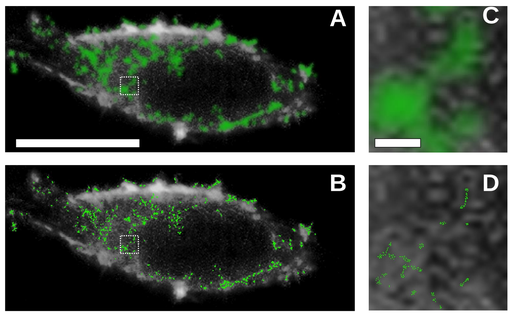

Fig. 7: Comparison of (A, C) optical diffraction-limited images and (B, D) super-resolution PHOTON images of single L-R complexes demonstrates superior super-resolution imaging capability of PHOTON. Zoom-in images of those squared in (A-B) are shown in (C-D). Scale bars are 10 µm in (A-B) and 0.5 µm in (C-D).

Summary

In summary, we have designed, synthesized, and characterized a mini-library of stable (non-aggregated) and purified NPs with various sizes, surface charges and chemical properties and designed early development zebrafish embryos as *in vivo* assays to study biocompatibility and toxicity of NPs. We found single NPs (1-100 nm) can passively diffuse into the embryos and create unique effects on embryonic development that are highly dependent upon size, dose and chemical property of the NPs and embryonic stages. We have developed imaging platform (e.g., DFOMS, PHOTON) to: (i) continuously and simultaneously image multiple single NPs *in situ* in real time at nm resolution for rational design of biocompatible NPs; (ii) study dependence of plasmonic optical properties of single NPs upon their size, shape, surface and chemical properties for rational design of single photonic imaging probes and arrays; (iii) probe efflux kinetics of single membrane transporters of single live cells in real-time at nm resolution for better understanding of smart sensing mechanisms and multi-drug resistance of membrane transporters; (iv) study size-dependent efficacy of drug nanocarriers; (v) detect and sense single protein molecules (tumor markers, cytokines) for molecular diagnosis; (vi) map single receptor molecules and its binding with receptors on single live cells, aiming to depict signaling transduction pathways in real-time. Our studies have offered powerful new tools and new insights into rational design of biocompatible and eco-friendly nanomaterials.

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