A novel biocompatible fluorescent nanoparticle enables enhanced live cell tagging and tracking of cancer cells

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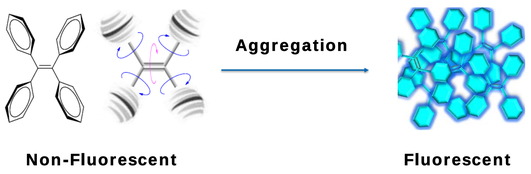
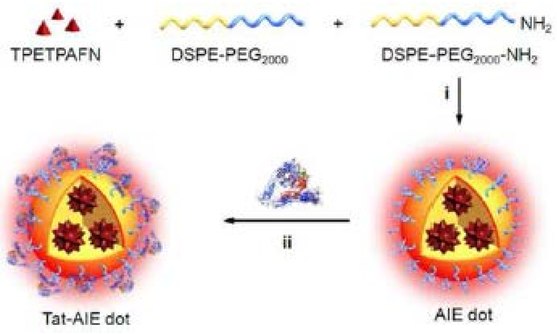
Introduction

Metastasis is the leading cause of cancer mortality. Metastasis is a multi-step process which includes local tumor cell invasion, cell migration into the vasculature, exit of cells from the circulation and colonization at the distal tissue sites. Long-term noninvasive cell tracking by fluorescent probes is of great importance to life science and biomedical engineering. Current methods used to fluorescently tag cancer cells have been limited by short signal duration, high background auto-fluorescence or lengthy cell line generation using GFP. We have developed a biocompatible fluorescent nanoparticle which relies on Aggregation Induced Emission (AIE) technology that are highly resistant to fluorescent signal quenching. These particles enable highly efficient live cell fluorescent tagging while retaining fluorescent signal for up to 10 days in vitro and 21 days in vivo. These nanoparticles will open new avenues in the development of fluorescent probes for following biological processes such as carcinogenesis.

Aggregation induced emission (AIE) molecules emit fluorescence in an opposite manner than other common fluorophores (Quantum Dots, GFP). Propeller-shaped AIE fluorogens are non-emissive in solutions but become highly fluorescent upon aggregate formation because the restriction of the intramolecular rotations (RIR). Due to these differences, AIE molecules have very high fluorescence intensities with minimal signal quenching. These properties make them optimal candidates for long interval live cell bioimaging experiments.



Fabrication of organic Tat-AIE dots includes encapsulation of the TPETPAFN AIE molecules within a DSPE-PEG200 outer shell with attached cell permeable TAT sequences.



TAT-AIE Dots have been commercialized by LuminiCell™ as LuminiCell Tracker™ Dyes

Methods

1) HeLa cells were cultured in 10% FBS media and transferred to a 8-well Lab-Tek chambered slide in a CO_2 incubator at 37° C until cells reach 80% confluence.
2) Prepare the LuminiCell Tracker™ labeling solution at 2-10 nM working concentration by diluting the stock LuminiCell Tracker™ solution using fresh growth medium.
3) Add 0.2-0.4 mL of labeling solution into each well to label cells. Incubate the cells at 37° C for ~1-4 hrs.
4) Wash the cells twice with growth medium.
5) Visualize the labeled cells using any suitable fluorescence microscopy preferred by the user or flow cytometry with compatible lasers/filters.

Results

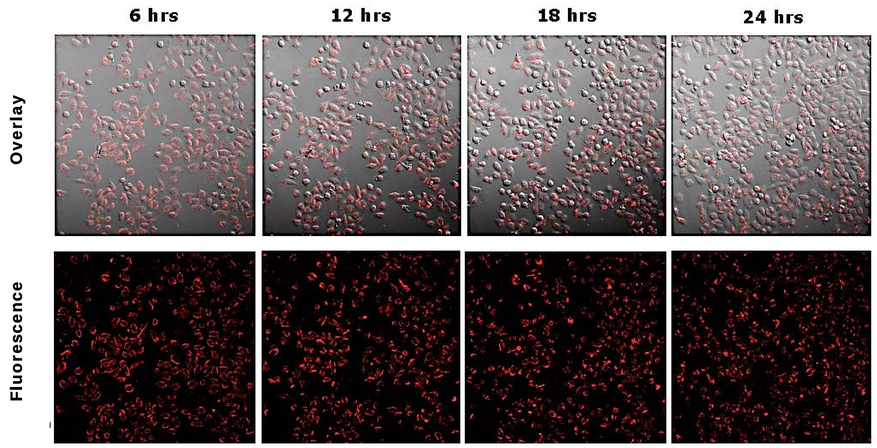


Figure 1. LuminiCell Tracker™ 670 Dyes are non-toxic to HeLa cells over a 24 hour time period while maintaining high fluorescent intensities

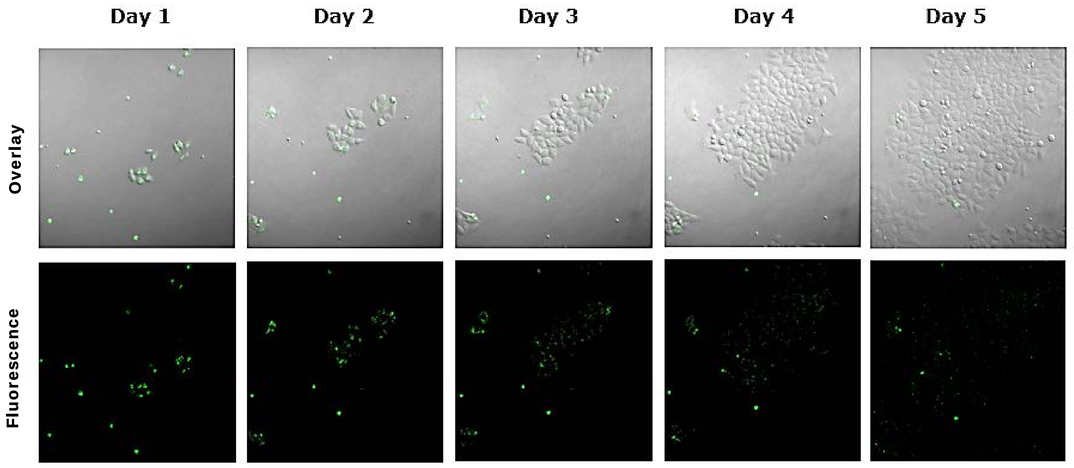


Figure 2. HeLa cells labeled with LuminiCell Tracker™ 540 Dye shows increased resistance to signal quenching over 5 days

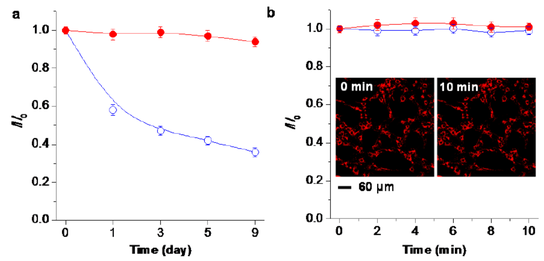


Figure 3. LuminiCell Tracker™ Dyes show increased resistance to photo bleaching in A) labeled HeLa cells over a 9 day time period and with B) 10 minutes of continuous laser exposure. Red dots represent LuminiCell Trackers™ and Blue dots represent Quantum Dots.

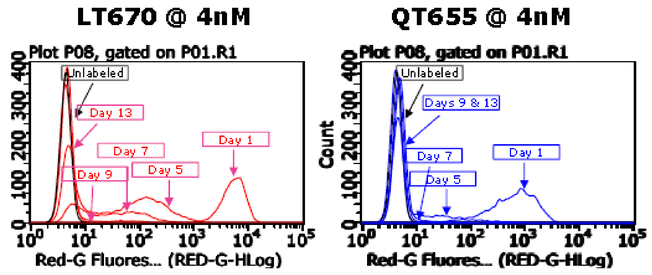


Figure 4. FACS analysis of LuminiCell Tracker™ (LT670) or Quantum Dots (QT655) labeled HeLa cells over a 9 day timecourse. LuminiCell Tracker™ labeled cells remain fluorescent for 7-9 days while Qdot labeled cells decrease fluorescence quickly after 2-3 days with zero emission at day 5.

Summary

The ability to image single-cell migration in real time is important to several research areas such as embryogenesis, cancer metastasis, stem cell therapeutics, and lymphocyte immunology. To overcome the shortcomings of inorganic QD-based direct cell labeling reagents, we developed photostable organic fluorescent dots with high quantum yield, bright FR/NIR emission and low cytotoxicity, which could act as a novel class of promising long-term cell tracing probes.