Electronic Supporting Information

# Red Blood Cell Membrane-Mediated Fusion of Hydrophobic Quantum Dots with Living Cell Membranes for Cell Imaging†

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## 1. Preparation of RBC-encapsulated QDs
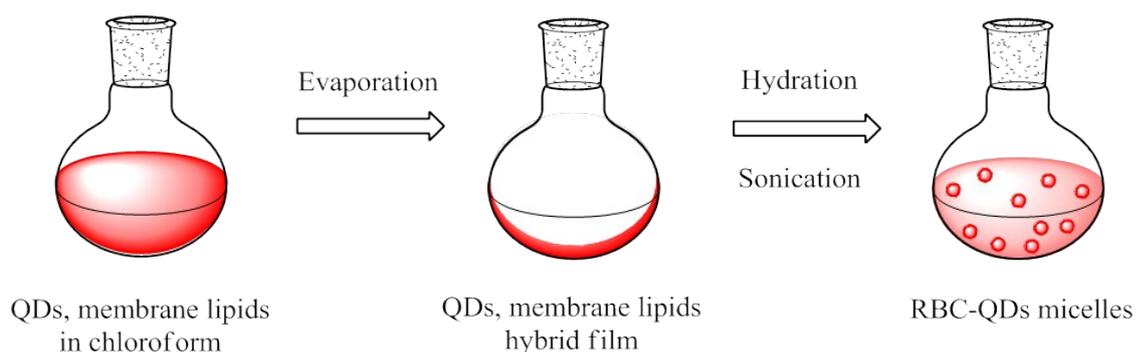


**Fig. S1** Methodology for the formation of QD encapsulated lipid micelles. A concentrated solution of QDs in chloroform was added to chloroform/ methanol solution dissolved with membrane lipids under vigorous stirring. Organic solvent evaporation during a course of heat treatment transferred the hydrophobic QDs to an aqueous phase. This interfacial process is driven by hydrophobic Vander Waals interactions between the primary alkane of the stabilizing ligand of octadecylamine and the secondary alkane of the membrane lipids, resulting in thermodynamically defined, interdigitated bilayer structures.

## 2. UV-vis absorption spectra of RBC-QDs
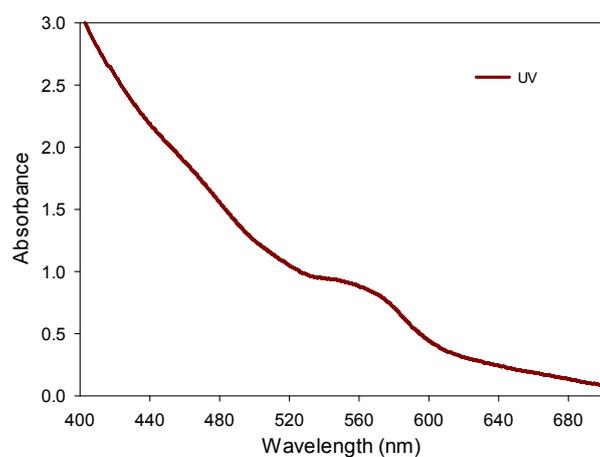


**Fig. S2** UV-vis absorption spectra of RBC-QDs

## 3. TEM images of hydrophobic QDs and RBC-QDs
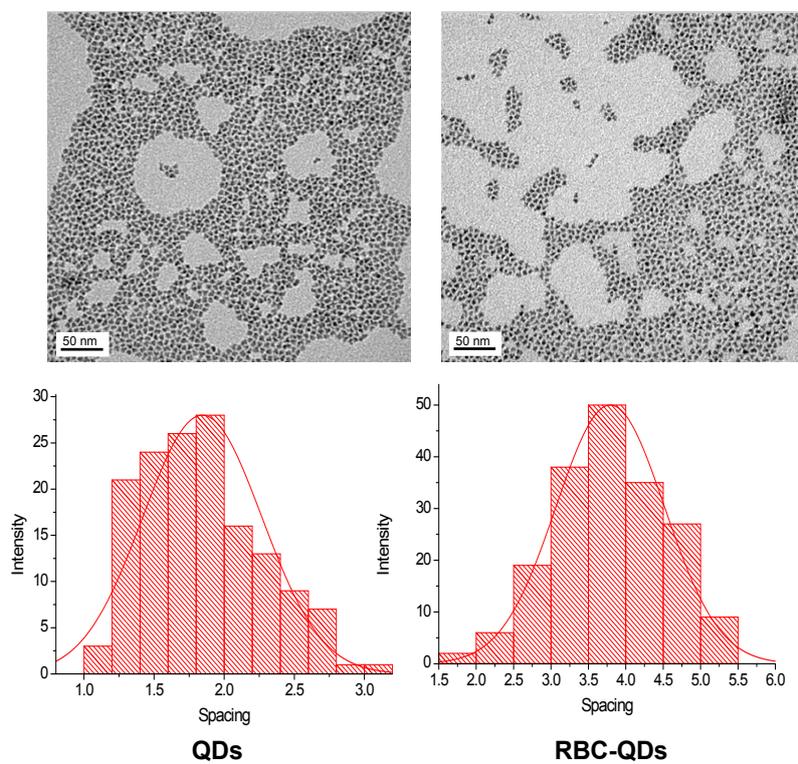


**Fig. S3** TEM images of hydrophobic QDs and RBC-QDs and the corresponding Gauss distribution curve of the particle spacing of them.
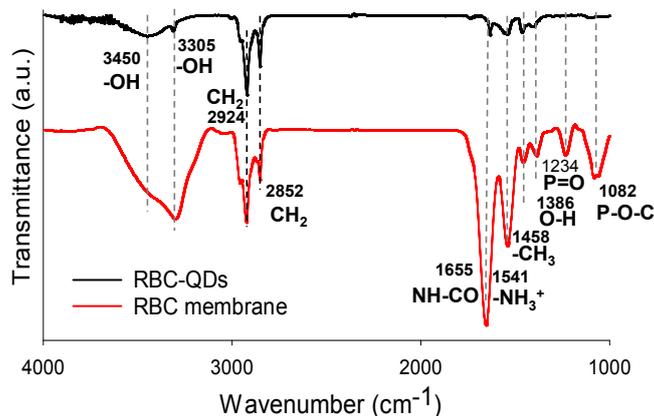
## 4. FTIR of RBC membrane and RBC-QDs.



**Fig. S4** FTIR of RBC membrane (red) and RBC-QDs (black).

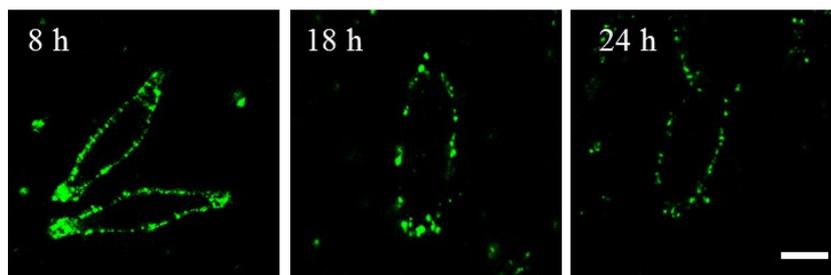## 5. Fluorescence confocal scanning imaging



**Fig. S5** Long-term imaging of RBC-QDs incubated with SMMC-7721 cell for 8 h, 18 h, and 24 h, respectively (left to right). Scale bar: 10 μm.

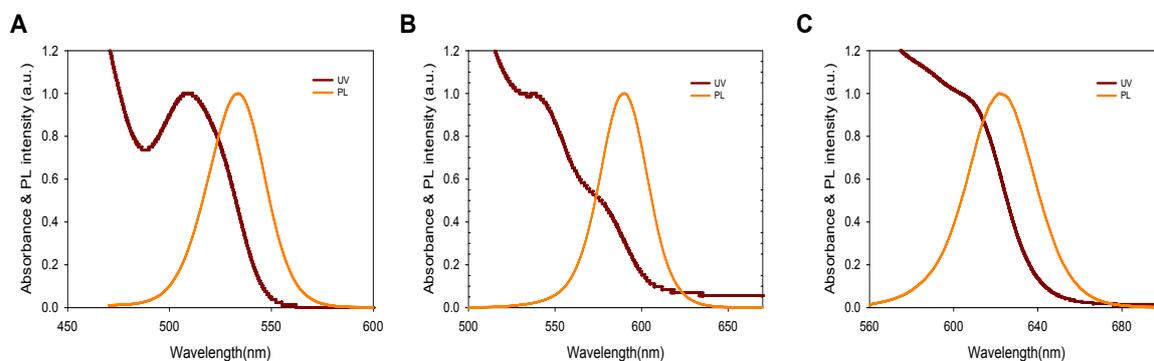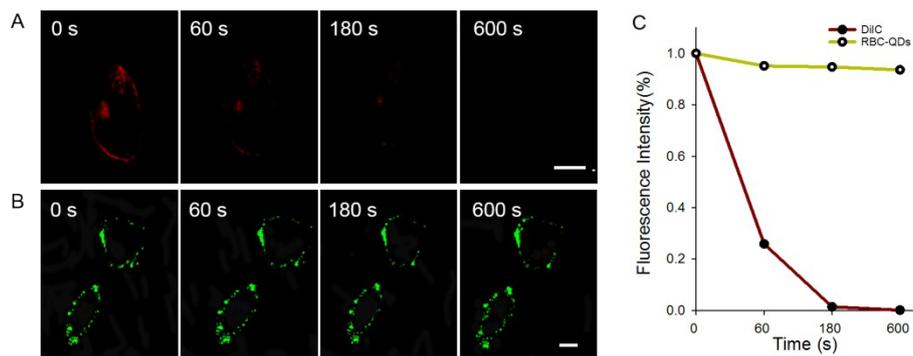## 6. UV-vis absorption and fluorescence emission spectra of three hydrophobic QDs



**Fig. S6** UV-vis absorption and fluorescence emission spectra of three hydrophobic QDs, (A): QDs530, (B): QDs585, and (C): QDs620.

## 7. Photostability of RBC-QDs as cell membrane marker



**Fig. S7** Photostability comparison of DiIC and RBC-QDs as cell membrane markers. Laser scanning confocal microscopy images of SMMC-7721 cells that were labeled with (A) DiIC and (B) RBC-QDs that were taken at 0 s, 60 s, 180 s, and 600 s upon continually intense excitation. (C) Corresponding fluorescence intensity curves of A and B. Scale bar: 10μm. Objective: 40×
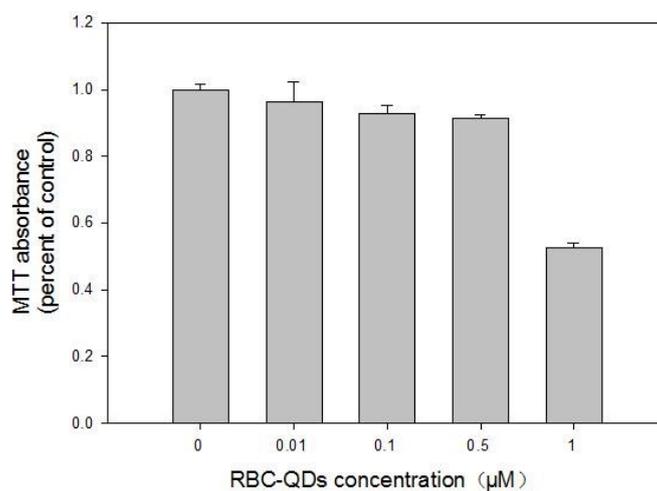


**Fig. S8. MTT assay for the QDs toxicity to cell.**

SMMC-7721 cells were seeded onto 96-well plates at a density of $7 \times 10^3$ cells per well in 200 μL of medium containing 10% fetal calf serum and incubated at 37 °C and 5% $CO_2$. After grown overnight, 200 μL of new medium containing various concentrations of RBC-QDs was added into cells. After incubated for 24 h, all cells were further incubated with fresh medium containing MTT (0.5 mg/mL) for 4 h. Then the medium was withdrawn and 150 μL of DMSO was added into each well to dissolve the precipitated formazan violet crystals at 37 °C for 10 min. The absorbance was measured at 490 nm by a multidetection microplate reader.

## 8. Single-nanoparticle imaging and tracking

Single-nanoparticle fluorescence measurements were performed on a total internal reflection fluorescence (TIRF) microscope (Nikon, TE-2000U inverted microscope) with a 60× TIRF oil objective. The fluorescence image was captured on a C9100-13 EMCCD (Hamamatsu). The fluorescent imaging data were analysed using Simple PCI 6 software. For single-nanoparticle tracking of QD fusion within the cell, movies of QDs were collected for more than 10 s. Single-spot trace extraction was performed using ImageJ (plugin SpotTracker 2D). The diffusion coefficients were calculated based on a mean square displacement (MSD) algorithm and custom script written in MATLAB was used to acquire them.

Single nanoparticle tracking is an effective technique to investigate the diffusion behaviors of individual nanoparticles and resolve their modes of motion, which could provide useful photo physical information on their microscopic environments and inherent structural features in liquid phase.[1] When an individual nanoparticle diffuses in free solution, the mode of its motion is merely pure diffusion; that is, Brownian motion. According to the classic Stokes-Einstein relation, the diffusion coefficient $D$, of a nanoparticle in the solution is described as follows.

$$D = kT / 3\pi\eta d \qquad (1)$$

Where $\eta$ is the pure solvent viscosity, $T$ is the absolute temperature, $k$ is the Boltzmann constant, and $d$ is the effective hydrodynamic diameter. From equation 1, we can know that $D$ is mainly related to $\eta$, $d$, and $T$. It means that under identical temperature and aqueous environment, $D$ is positively correlated to $d$, the effective hydrodynamic diameter of the QDs micelles themselves. As a nanoparticle moves near the surface, it tends to be found farther and farther away from its starting point, as described for a random walk process. The spreading could be figured from the captured image time series as the mean square displacement (MSD) of each nanoparticle.[2, 3] MSD functions can be calculated by averaging over all pairs of points with a given time lag with the following relation.

$$MSD = \langle r^2(t) \rangle = \langle \frac{1}{N} \sum_{i=0}^{N} (r_i(t) - r_i(0))^2 \rangle \qquad (2)$$

$$\langle r^2 \rangle = 2D\Delta t \qquad (3)$$

Where $r_i$ is the nanoparticle coordinate on frame $i$, $N$ is the total number of steps in the trajectory, $\triangle t$ is the interval time between each frame, and $t$ is the time interval over which the MSD is calculated. The MSD function was calculated for each step from 0 to $i$ pairs of points, which has in simulations been proven to be enough to estimate the short-term diffusion coefficient with high accuracy.[4] The diffusion

coefficient $D$ could then be estimated by fitting the first several points in the MSD function versus time using the relation for a two-dimensional random walk. (Fig. S9)
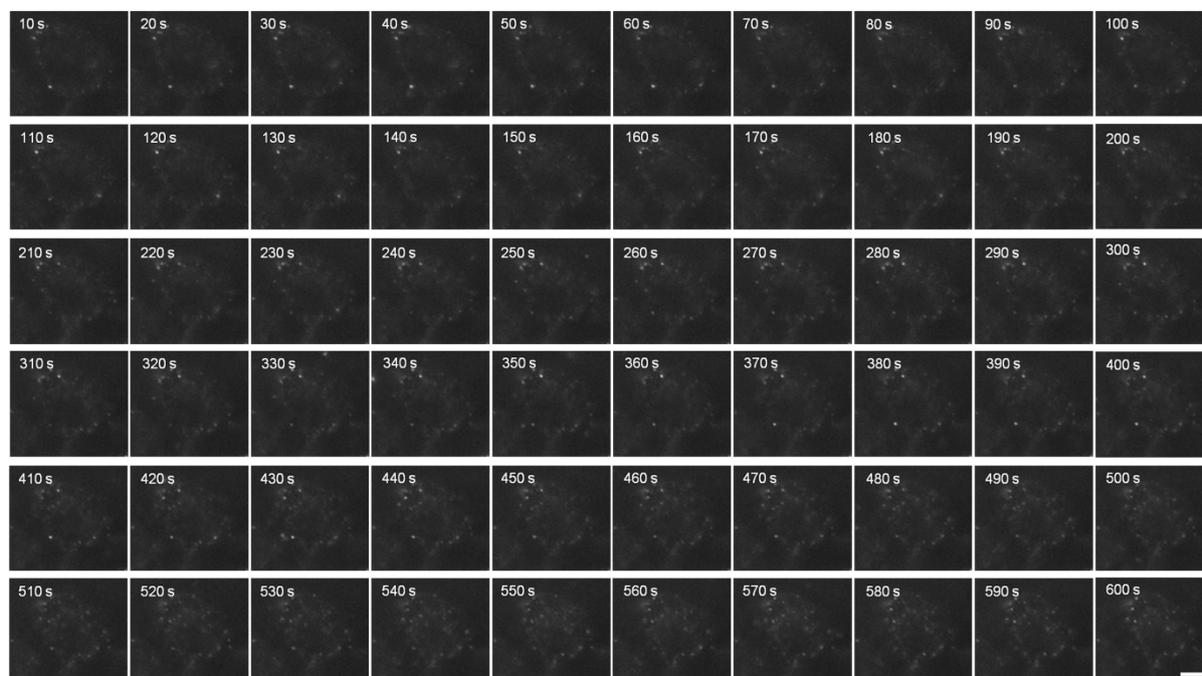


**Fig. S9** Single-nanoparticle RBC-QDs on a cell surface for 10 min at an elevated temperature. It was observed that a population of QDs accumulated on the cell membrane and then gradually shifted toward the cell center. The migration of QDs was triggered by cell movement and it was also observed that an obvious shrinkage and contraction occurred in the cell. Fig. 4D demonstrated a representative diffusion trajectories of individual RBC-QDs in a velocity of $3.4 \times 10^{-3}$ μm s$^{-1}$. Bar: 5 μm.

**References**

1    R. A. Sperling, T. Liedl, S. Duhr, S. Kudera, M. Zanella, C. A. J. Lin, W. H. Chang, D. Braun and W. J. Parak, *J. Phys. Chem. C,* 2007, **111**, 11552-11559.
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3    M. K. Cheezum, W. F. Walker and W. H. Guilford, *Biophys. J.,* 2001, **81**, 2378-2388.
4    M. J. Saxton, *Biophys. J.,* 1997, **72**, 1744-1753.