Supporting Information

Study of Diffusion Dynamics and Concentration Distribution of Gold Nanospheres (GNSs) without Fluorescent Labeling inside Live Cells Using Fluorescence Single Particle Spectroscopy

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MEMFCS method. According to the MEMFCS model developed by Maiti et al.(P. Sengupta, K. Garai, J. Balaji, N. Periasamy and S. Maiti, Biophys J., 2003, 84, 1977-1984.), MEMFCS was used to study the diffusion dynamics of fluorescent GNPs within live cells. The autocorrelation function describing the translational diffusion of fluorescent GNSs in live cells can be expressed as

$$G_D(\tau) = \frac{1}{N} \times \frac{1}{\left(1 + \left(\frac{\tau}{\tau_D}\right)^{\alpha}\right)} \times \frac{1}{\sqrt{1 + \left(\frac{\omega_0}{z_0}\right)^2 \times \left(\frac{\tau}{\tau_D}\right)^{\alpha}}} \quad (1)$$

Where ω_0 and z_0 are the lateral and axial radii of the confocal volume. τ_D is the average time of fluorescent molecules or nanoparticles through the volume. N is the average number of fluorescent molecules or particles in the volume. α represents the degree of anomalous diffusion behavior.

Then, G (τ) in eq. 1 can be rewritten to obtain a continuous distribution of diffusion time using MEM-RLSCS as

$$G(\tau) = \int \alpha(\tau_D) \cdot (1 + \frac{\tau}{\tau_D})^{-1} \cdot (1 + (\frac{\omega_{xy}}{\omega_z})^2 \cdot \frac{\tau}{\tau_D})^{-1/2} d\tau_D \quad (2)$$

In this MEM-RLSCS model, the diffusion time τ_D is considered to be a variable and $\alpha(\tau_D)$ is the amplitude associated with τ_D. The distribution of diffusion times (α (τ_D) vs. τ_D) is obtained by an optimal fitting based on the maximum entropy method by minimizing χ^2 as well as maximizing entropy S when the diffusion times of the different components are involved in a given focal volume.

χ^2 is defined as

$$\chi^2 = \frac{1}{M}\sum_{i=1}^{M} r_i^2 \qquad (3)$$

$$r_i = \frac{G^c(\tau_i) - G^e(\tau_i)}{\sigma_i} \qquad (4)$$

In eq. 3 M is the number of FCS data points. σ_i is the inverse of weight for the i_{th} data. $G^c(\tau_i)$-$G^e(\tau_i)$ is the differenced value of calculated value using eq. 2 and the experimental value. For a good fitting, χ^2 is approximately equal to unity when M is sufficiently large.

S is defined as

$$\begin{aligned} &\text{Max } S = -\sum_{i=1}^{n} p_i \ln p_i \\ &p_i = \frac{\tau_{Di}^{12} N_i}{\sum_{i=1}^{n} \tau_{Di}^{12} N_i} \\ &s.t. \sum_{i=1}^{n} p_i = 1 \end{aligned} \qquad (5)$$

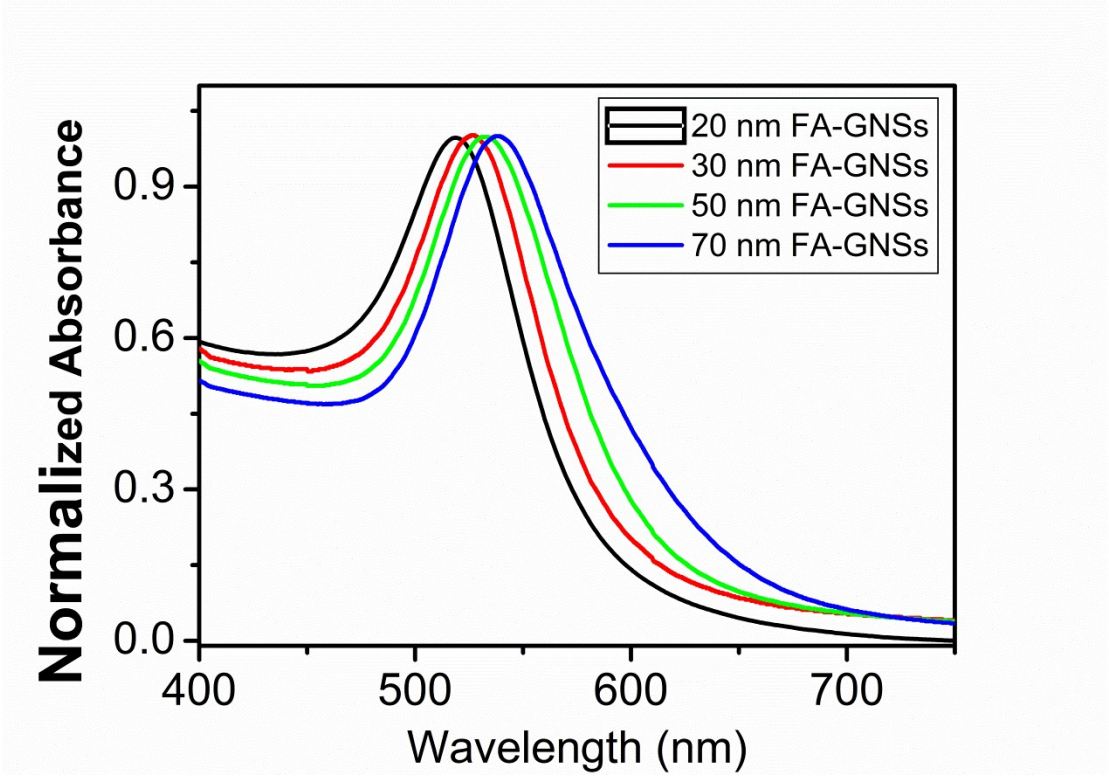

Figure S1.UV-vis absorption spectra of FA-GNSs

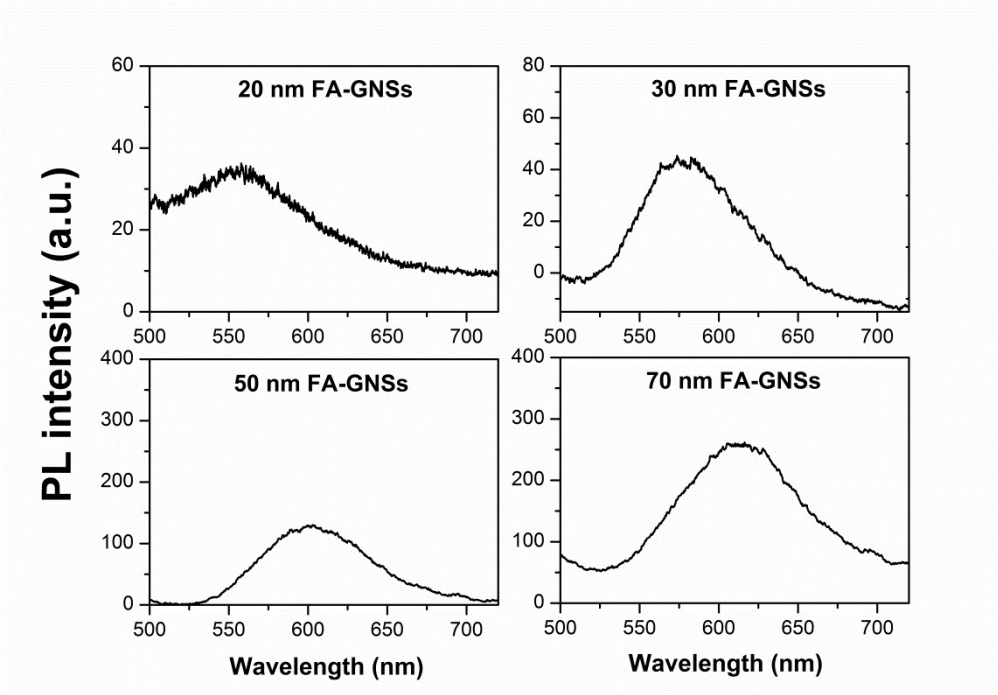

Figure S2. PL spectra of FA-GNSs

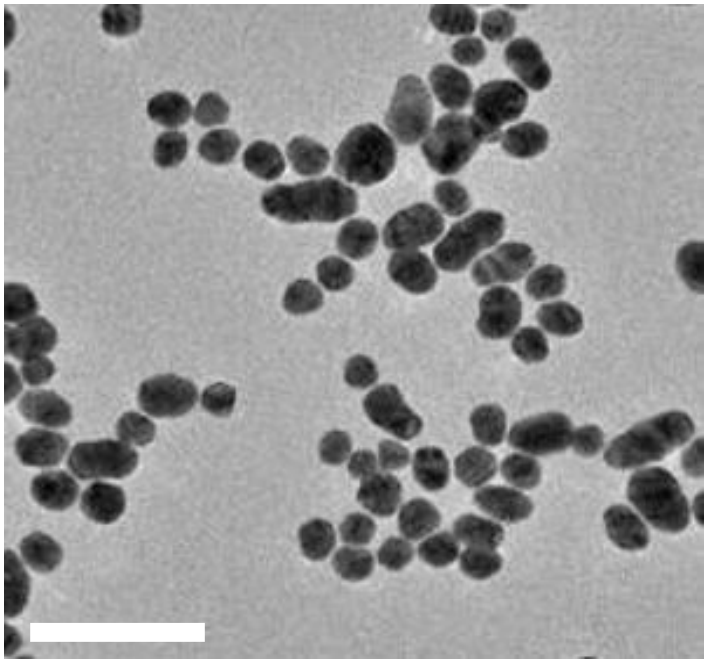

Figure S3. The TEM images of GNSs. Scale bar：100 nm

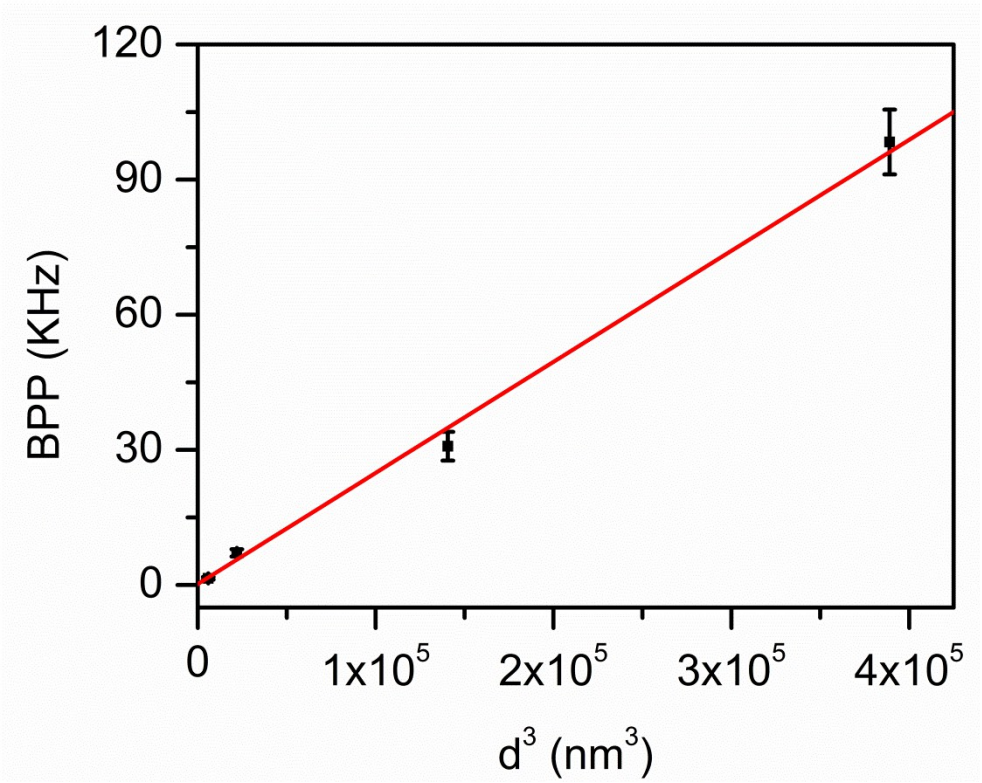

Figure S4. Linear relationship between brightness per particle (BPP) and the third power of GNSs' diameters (d^3)

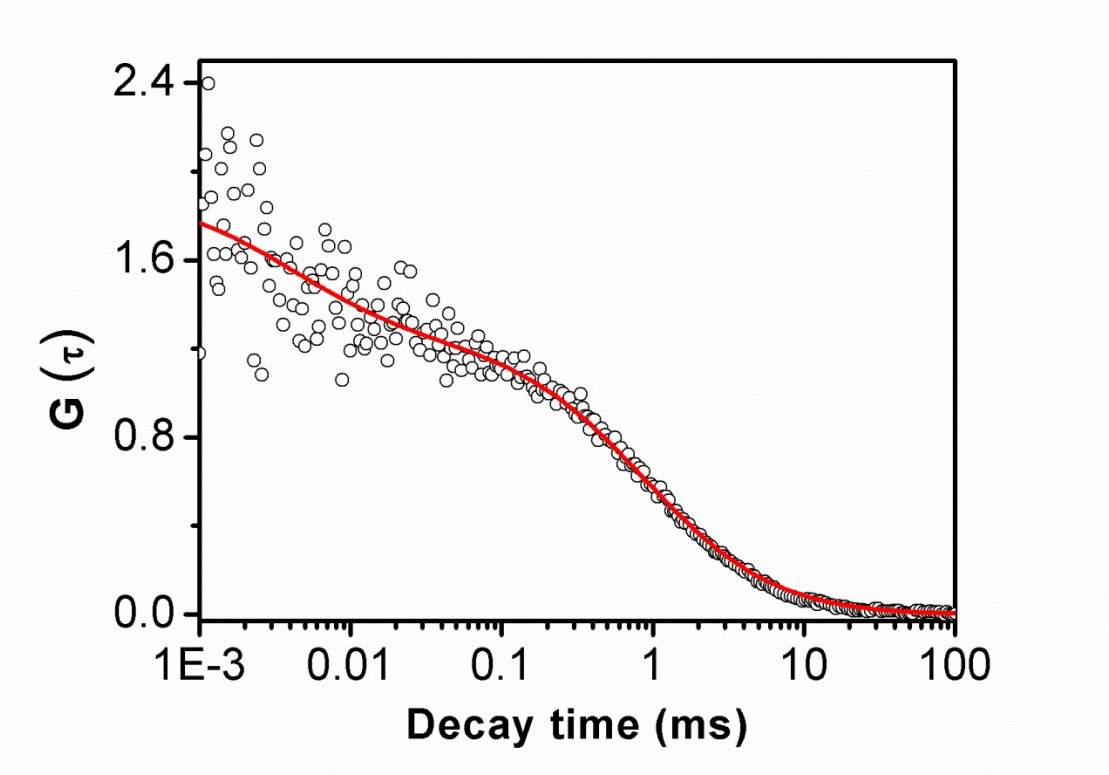

Figure S5. The typical fluorescence autocorrelation spectroscopy (FCS) curve of 30 nm FA-GNS in the pure water. The laser intensity is about 3 mW for excitation.

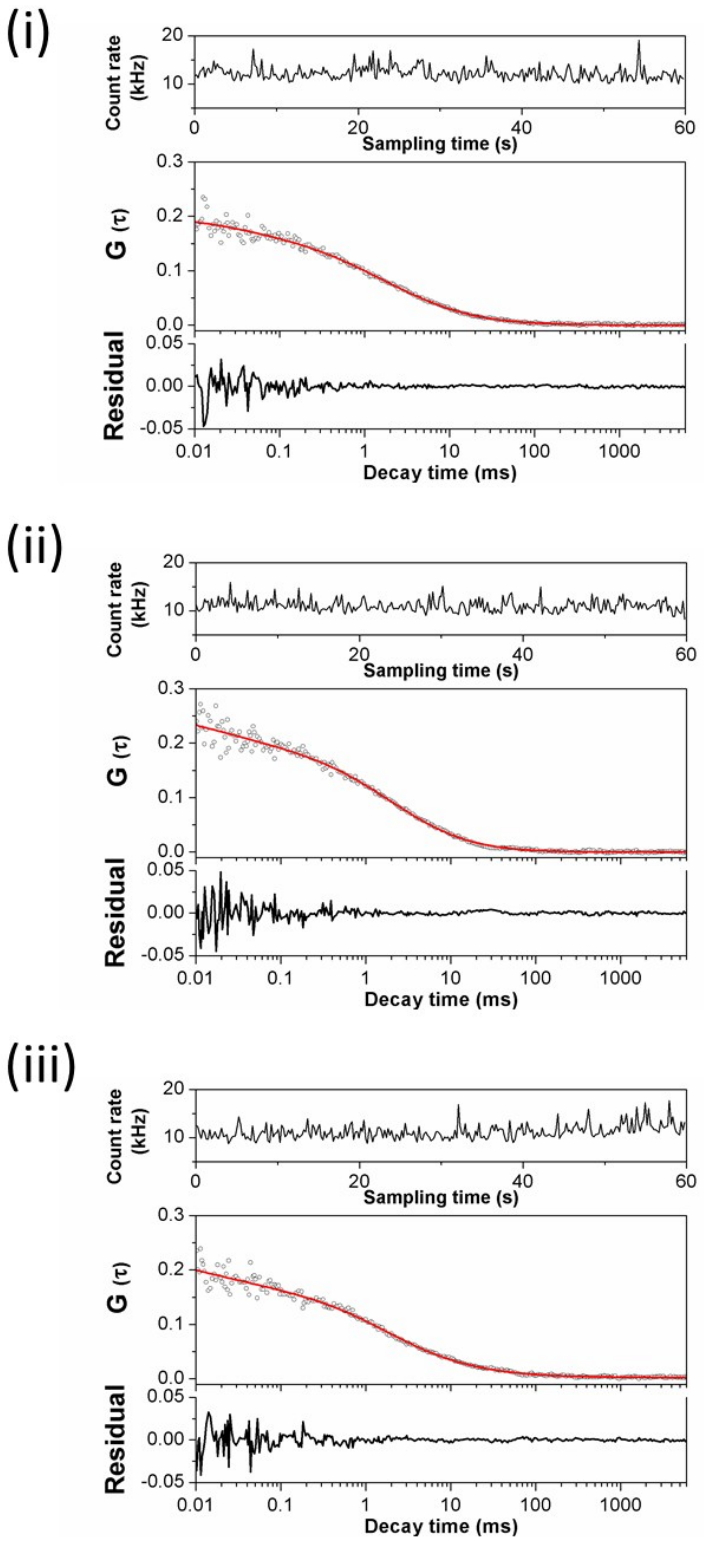

Figure S6. The fluorescence (count rate) fluctuation (upper), raw FCS and fitting curves from MEMFCS analysis (middle, red line), and the fitting residual curve (lower) of FA-GNSs within HeLa cell successively measured for three times at one selected site.

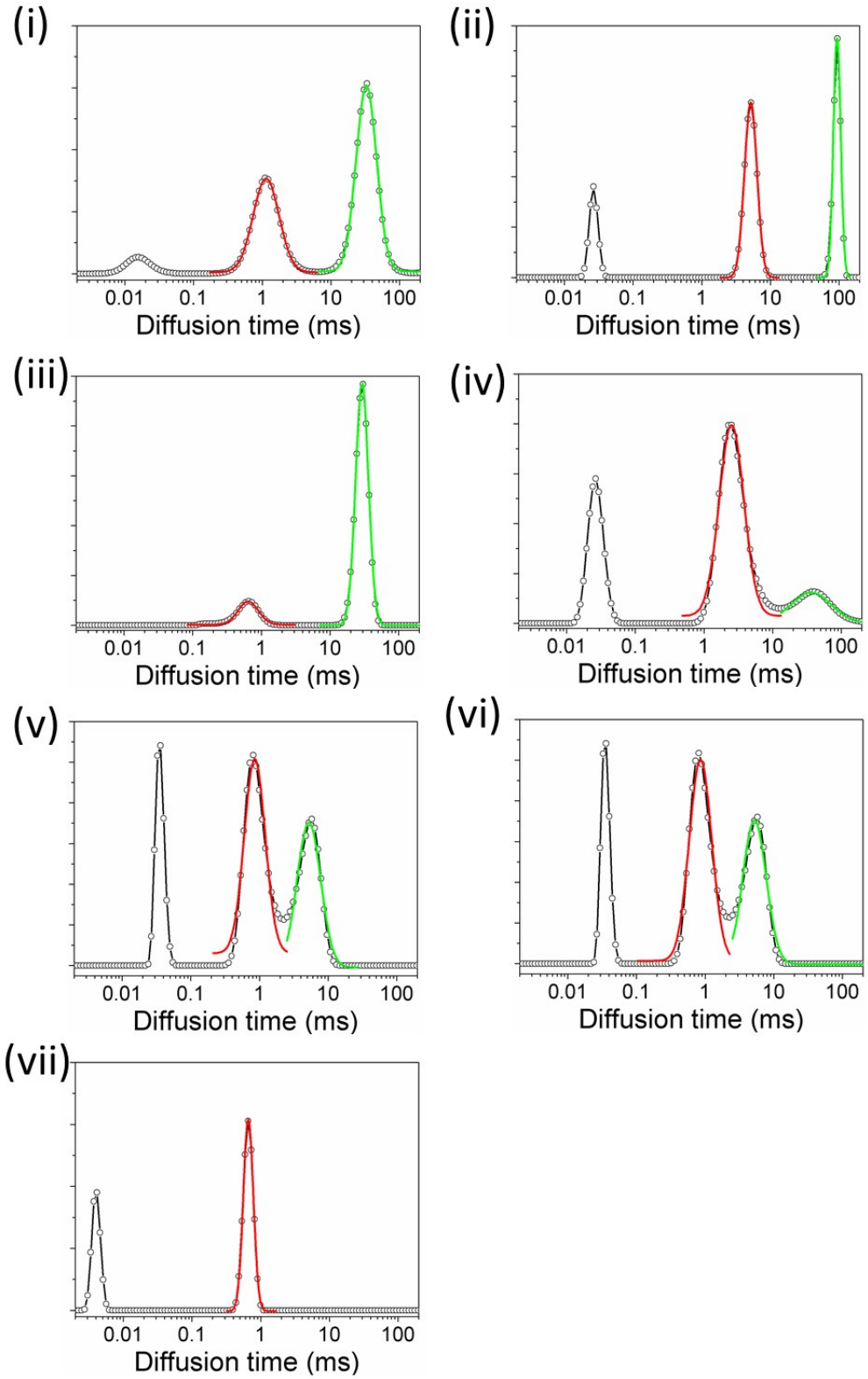

Figure S7. The diffusion time distribution of GNSs within live cells (i-vi) measured from the six intracellular areas and that of GNSs in the ultrapure water (vii), using MEMFCS method.

Table S-1 The concentration of GNSs determined by FCS

Sample No.	UV-vis/ nM	FCS/ nM	RSD / %
1	0.8	1.3	1.9
2	1.7	2.0	1.7
3	3.3	3.4	0.8
4	5.0	5.1	1.4
5	6.6	6.2	1.1