# Electronic Supplementary Information

# Formation of fluorescent platinum nanoclusters using hyper-branched polyethylenimine and their conjugation to antibody for bio-imaging

*Xin Huang,*[a] *Hidekazu Ishitobi,*[a, b] *and Yasushi Inouye*[*a, b, c]

[a]Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565-0871, Japan.

[b]Department of Applied Physics, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan.

[c]Photonics Advanced Research Center, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan.

[*]Corresponding author:

Yasushi Inouye

Email: ya-inoue@ap.eng.osaka-u.ac.jp

TEL: +81-6-68794615

FAX: +81-6-68794619

## Characterization.

*pH Measurement:* The pH of the samples was measured by pH meter F-52 (HORIBA, Ltd. Japan) at 25 ºC.

*DLS Measurement:* The average hydrodynamic size of the samples was measured by a high-performance two angle particle and molecular size analyzer (Zetasizer Nano ZS) using dynamic light scattering with 'NIBS' optics (DLS, Malvern Instruments, United Kingdom) at 25 °C.

*UV/Vis Spectrophotometric Analysis:* A UV-2450 spectrophotometer (Shimadzu Corporation, Japan) was used to measure the quantum yields (QYs).

*Photoluminescence Spectrophotometric Analysis:* The excitation and emission spectra of Pt NCs were measured with a FP-6200 Spectrofluorometer (Shimadzu Corporation, Japan) equipped with a high-power Xenon lamp.

*Transmission Electron Microscopy (TEM):* TEM was performed at room temperature with JEM-1011 (JEOL, Japan), and the size-distribution of Pt NCs@PEI was analyzed by ImageJ 1.47v software.

*Quantum Yield Measurement:* The photoluminescence (PL) QY of a compound is defined as the fraction of molecules that emit a photon after direct excitation by the source.[1] This quantity is not the same as the number of emitted photons that escape a bulk sample divided by the number of absorbed photons, although in many instances the two quantities are nearly equal.

Equation 1 was used to measure the relative PL QY:

$$\Phi_{unk} = \frac{A_{std}}{A_{unk}} \times \frac{F_{unk}}{F_{std}} \times \frac{{n_{unk}}^2}{{n_{std}}^2} \times \Phi_{std} \qquad (1)$$

Φ = QY; unk = unknow sample; std = standard; n = refractive index of solvent; A = absorption at the selected excitation wavelength; and F = integrated fluorescence signal in the emission region. To calculate the QY of Pt NCs@PEI, rhodamine 6G (Φ = 0.95) was employed as the standard and a UV-2450 spectrophotometer (Shimadzu Corporation, Japan) was used. All samples were diluted to ensure optical densities of less than 0.02 in order to reduce the error. Emission spectra were recorded on a FP-6500 fluorescence spectrometer under the excitation of 500 nm light.

## Conjugation of Qdots to Antibody.

A common EDC/Sulfo-NHS coupling reaction was employed to conjugate Qdots@COOH to the antibodies. EDC (4.0 μL, 1 mM) and sulfo-NHS (4.0 μL, 1 mM) was added into 1 mL Qdots@COOH (200 nM) and the mixture was activated for 30 min at room temperature. The molar ratio of EDC/NHS/ Qdots was 20:20:1. Subsequently, anti-CXCR4-Ab (20 μL, 1 mg/mL) was introduced into the mixture, which was stirred for another 2 h. Finally, the Qdots@COOH-CXCR4-Ab solution was incubated at 4 °C overnight. The purification and storage methods were the same as above for Pt NCs@PEI-(anti-CXCR4-Ab).

**Figure S1-S7.**

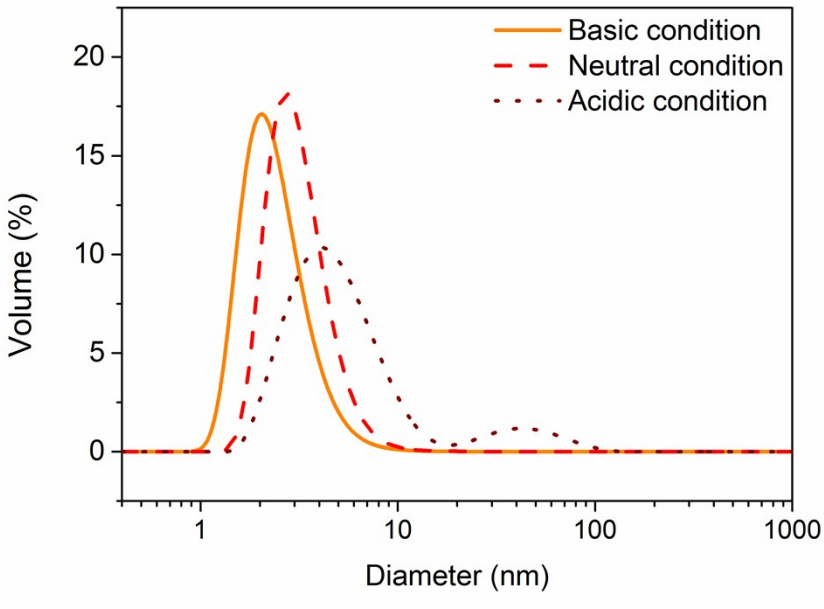


**Fig. S1** DLS spectrum of Pt NCs@PEI at various pH conditions during the reduction process.

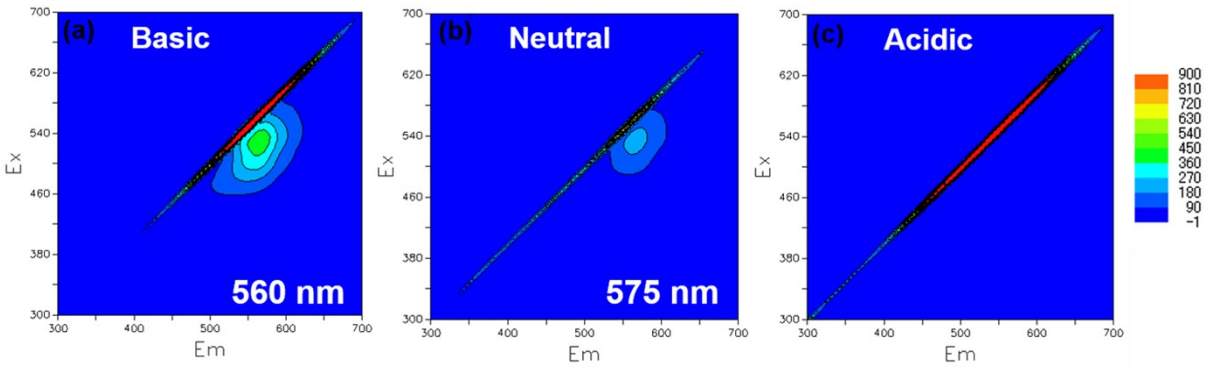


**Fig. S2** Excitation and emission matrix (EEM) spectra of Pt NCs@PEI synthesized under the basic (a), neutral (b), acidic (c) condition fixing the molar ratio between Pt ions and reducing agent at 1:25.

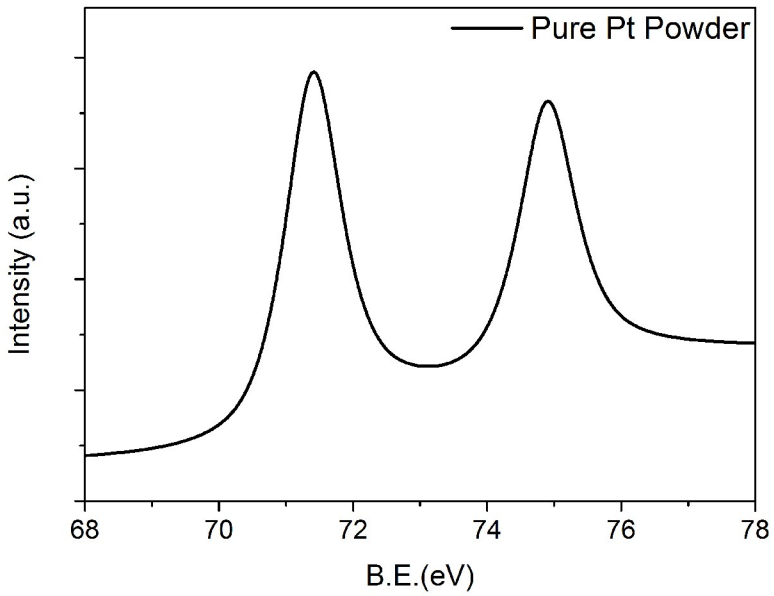


**Fig. S3** XPS of the pure Pt powder.

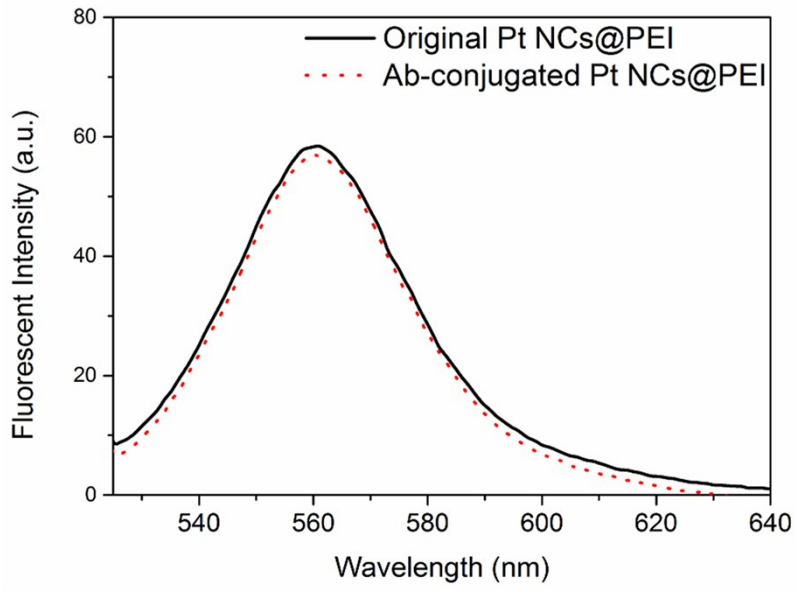


**Fig. S4** Fluorescence spectra of Pt NCs@PEI (black solid line) and Pt NCs@PEI-(anti-CXCR4-Ab) (red dotted line).

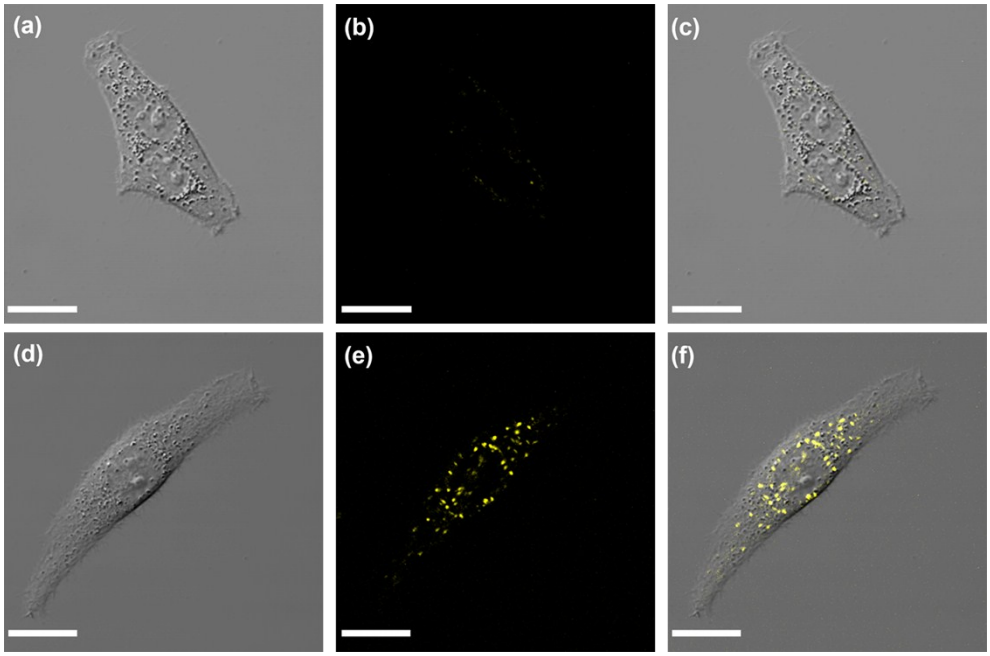


**Fig. S5** (a) Differential interference contrast (DIC) image, (b) laser confocal fluorescent microscopic image, and (c) merging of the two of living HeLa cells labeled with Pt NCs@PEI. (d) DIC image, e) fluorescent image, and (f) merging of the two images of living HeLa cells labeled with Pt NCs@PEI-(anti-CXCR4-Ab) conjugates. Scale bars, 20 μm.

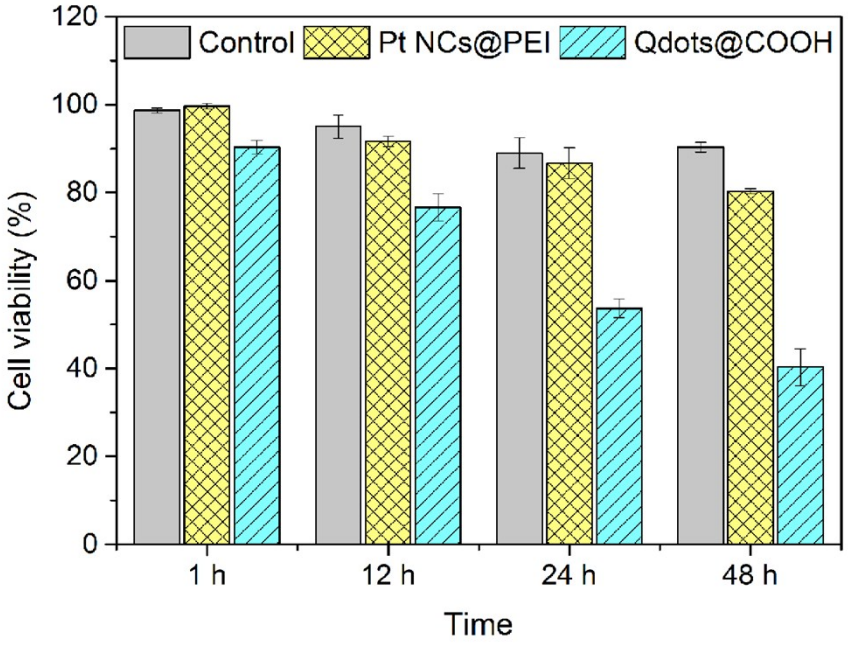


**Fig. S6** Cell viability of HeLa cells incubated with no label (control group), Pt NCs@PEI (50 nM) or Qdots@COOH (50 nM) at different incubation times.

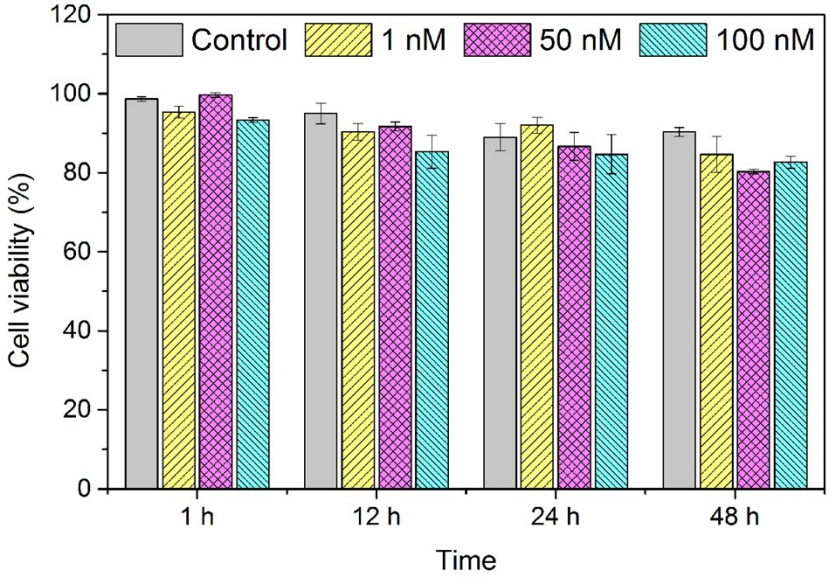


**Fig. S7** Cell viability of HeLa cells incubated with Pt NCs@PEI at various concentrations and incubation times.

## References.

1 G. A. Crosby and J. N. Demas, *J. Phys. Chem.*, 1971, **75**, 991-1024.