Nanoclusters

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Fluorescent Platinum Nanoclusters: Synthesis, Purification, Characterization, and Application to Bioimaging**

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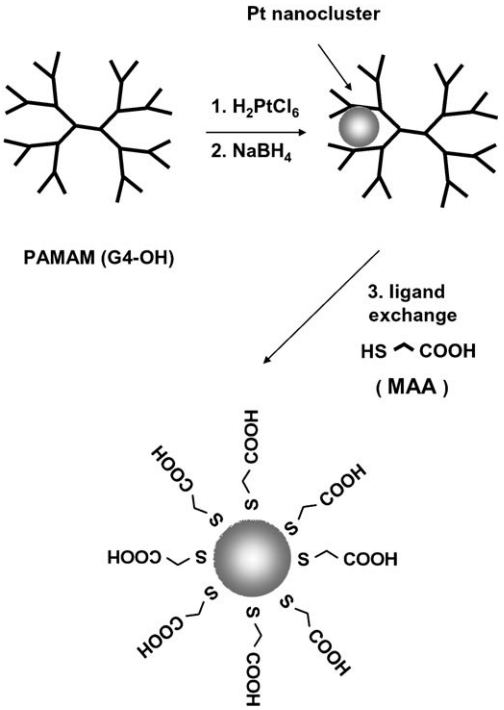
Noble-metal nanoclusters[1–8] consisting of several atoms have been gaining much attention as novel fluorescent markers[7,8] owing to their optical properties, which include size-dependent emission wavelength and discrete electronic state, features that are similar to semiconductor quantum dots. However, their smaller size and lower cytotoxicity in some ways make metal nanoclusters superior. Dickson and co-workers reported the synthesis and characterization of gold[1] and silver[4,7] nanoclusters and successful bioimaging of actin filament labeled with these nanoclusters,[7a] while Lin et al. used peptide-conjugated gold nanoclusters as nuclear targeting and intracellular imaging probes.[8a] However, among the noble-metal clusters, platinum clusters have not yet been used as bioimaging probes except as a reducing catalyst.[9] Herein, we describe water-soluble platinum nanoclusters that are less cytotoxic and emit a brighter fluorescence at 470 nm than other fluorescent nanomaterials, such as gold clusters.[2] The synthesis of such platinum nanoclusters is achieved by a simple chemical reduction; they are purified by size-exclusion high performance liquid chromatography (HPLC).

The size of the noble-metal nanoclusters is crucial because it affects the photoluminescence wavelength.[1a,b,2,3a,4a,5a] This parameter can be regulated by a macromolecule-based template such as a dendrimer.[1,8a,b] One example is the fourth-generation polyamidoamine dendrimer (PAMAM (G4-OH)), which has been used as a molecular template in the synthesis of fluorescent gold nanoclusters.[1,8a,b] This dendrimer has nanometer-scale size and a uniform structure comprising an internal core and an external shell. Since the internal core contains tertiary amines that can form coordination bonds with the metallic ions, PAMAM dendrimers can trap these metal ions[10] to form metal nanoclusters. Herein, we apply PAMAM (G4-OH) to synthesize Pt nanoclusters. Electronspray ionization (ESI) mass spectrometry confirmed that the synthesized nanoclusters were atomically monodispersed Pt_5.

Platinum nanoclusters were prepared by reducing H_2PtCl_6 (6.0 μL, 0.5 M) with $NaBH_4$ (3.0 μmol) in the presence of PAMAM (G4-OH) (0.5 μmol, 71.4 mg; Scheme 1). $NaBH_4$ was slowly added to the mixture under continuous stirring. To complete the reaction, the mixture was stirred for two weeks. Large Pt colloidal nanoparticles were removed by ultracentrifugation (Optima MAX-XP Benchtop Ultracentrifuge, Beckman Coulter, Inc.; 100000 G) for 30 min at 4 °C. The supernatant emitted a strong blue fluorescence under UV light (365 nm) irradiation, indicating Pt nanoclusters. However, Bard and co-workers have shown that simple oxidation of PAMAM (G4-OH) with $(NH_4)_2S_2O_8$ can also produce species that emit blue photoluminescence.[11] Therefore, we purified the Pt nanoclusters using size-exclusion HPLC (see the Experimental Section).



Scheme 1. Pt nanocluster synthesis and ligand exchange with mercaptoacetic acid (MAA).

To do this, mercaptoacetic acid (MAA) was added to the supernatant to replace PAMAM (G4-OH) as the ligand for

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the Pt nanoclusters (Scheme 1). The supernatant with added MAA was separated into several fractions using size-exclusion HPLC, and the fluorescence spectrum and lifetime of each fraction were measured.[12] The fraction obtained with 37 to 39 min retention time (Figure 1a) showed blue fluorescence at 470 nm (Figure 1b) and a fluorescence lifetime of (8.8 ± 0.5) ns, while another fraction from 48.5 to 51 min showed a lifetime of (6.3 ± 0.5) ns (Figure 1c). Since a (6.3 ± 0.5) ns lifetime corresponds to deliberately oxidized PAMAM (G4-OH), our results suggest that (8.8 ± 0.5) ns lifetime fractions contained purified Pt nanoclusters.

To characterize Pt nanoclusters, inductively coupled plasma mass spectrometry (ICP-MS) was performed for the 37 to 39 min retention time fraction, and only Pt (871.64 $\mu g L^{-1}$) was found to be present. The molecular weight was determined by ESI mass spectrometry upon dissolving the sample in a 50% (v/v) water/methanol system.[5h] The ESI data showed a main peak at $m/z = 1712$, which was assigned to $Pt_5(MAA)_8$ (MW = 1712.3, Figure 2) and reveals that the synthesized nanoclusters were atomically monodisperse Pt_5. In Figure 1a, we noticed that PAMAM (G4-OH) was eluted more slowly than the Pt nanoclusters although its molecular weight is larger than that of the latter. This can be explained by the electrostatic adsorption of PAMAM (G4-OH) on the silica-based column[13] of our size-exclusion HPLC, resulting in a longer elution time for PAMAM (G4-OH) than expected. A comparison of this elution time with standard samples (e.g. *p*-amino benzoic acid, MW = 137, and aprotinin, MW = 6511) strongly supports this explanation. Consequently, these results show that the size-exclusion HPLC technique can be used to purify Pt nanoclusters from PAMAM (G4-OH).

Fluorescent probe brightness is crucial for obtaining well-defined images with a high signal-to-noise ratio. We evaluated the absolute quantum yields (QY) of the synthesized Pt_5 nanoclusters using a quantum yield measurement system (C10027, Hamamatsu Photonics). Quantum yield is given by $QY = PN_{em}/PN_{ab}$, where PN_{em} and PN_{ab} are the number of emitted and absorbed photons by the fluorescent particles, respectively. The QY for the Pt_5 nanoclusters was found to be 18% in water, while that for another blue photoluminescent species, ZnSe quantum dots in chloroform, was 5%.[14] On the other hand, we measured the absolute QY of newly synthesized Au_8 nanoclusters[15] and found that the QY is 3.3%[2] in water. Absolute QY measurements show that the fluorescence of Pt_5 nanoclusters is at least three times brighter than that of ZnSe quantum dots and Au_8 nanoclusters.

Next, we investigated the capability of Pt_5 nanoclusters to act as fluorescent probes for live cell imaging. First, we conjugated the $Pt_5(MAA)_8$ with Protein A using a 1-[(3-dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC)/*N*-hydroxysulfosuccinimide (Sulfo-NHS) coupling reaction. We bound $Pt_5(MAA)_8$-(Protein A) to an anti-chemokine receptor antibody (anti-CXCR4-Ab) through the Fc moiety of the antibody (Scheme 2).[16] After the introduction of $Pt_5(MAA)_8$-(Protein A)-(anti-CXCR4-Ab) (57 nM) into HeLa cells, the HeLa cells were incubated for 5 min prior to cell imaging. Figure 3a shows a confocal fluorescence image of HeLa cells labeled with $Pt_5(MAA)_8$.

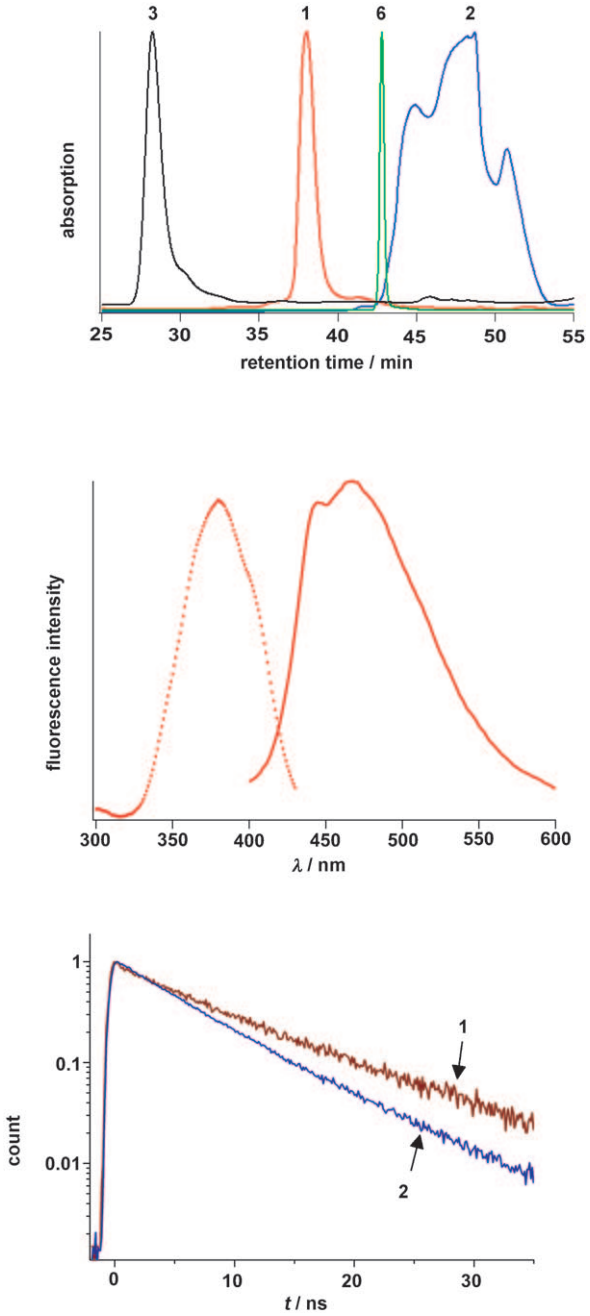


Figure 1. a) Size-exclusion HPLC chromatogram of the samples 1) (MAA)–Pt nanoclusters (red) and 2) PAMAM (G4-OH) (molecular weight = 14279; blue), and of standard samples 3) aprotinin (molecular weight = 6511; black) and 4) *p*-amino benzoic acid (molecular weight = 137; green). HPLC was monitored by UV absorption at 290 nm. b) Excitation (dashed line) and emission spectra (solid line) of Pt_5 nanoclusters in water. c) Fluorescence lifetime of Pt_5 nanoclusters. The fluorescence lifetimes of Pt_5 nanoclusters (1) and PAMAM (G4-OH) (2) were obtained by single exponential fitting ((8.8 ± 0.5) and (6.3 ± 0.5) ns, respectively).

Because chemokine receptors are highly expressed in tumor (HeLa) cells,[17] blue fluorescence (470 nm) was observed on cell membranes where the receptors were exhibited. No fluorescence signal was detected in a control sample labeled without $Pt_5(MAA)_8$ (Figure 3b), which rules out any auto-

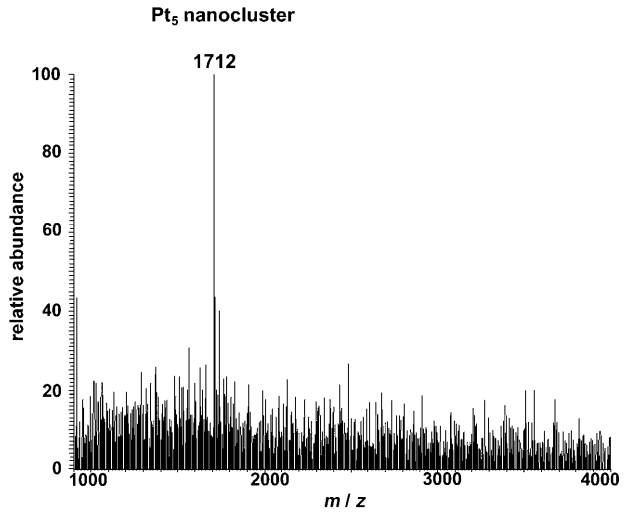
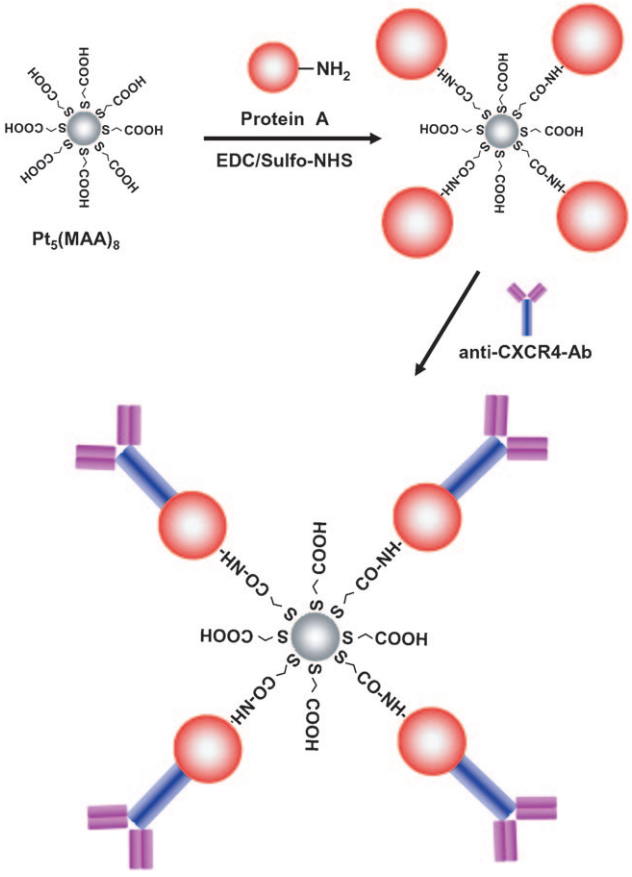


Figure 2. ESI mass spectrum of Pt_5 nanoclusters. The peak $m/z = 1712$ shows that nanoclusters consist of five platinum atoms.



Scheme 2. Preparation of $Pt_5(MAA)_8$-(Protein A)-(anti-CXCR4-Ab).

fluorescence at 405 nm excitation. To examine the specific binding of $Pt_5(MAA)_8$-(Protein A)-(anti-CXCR4-Ab) to the chemokine receptor, chemokine receptor negative CHO-K1 cells were used as a negative control.[18] In Figure 3c, no fluorescence signal was observed, thus indicating that CHO-K1 cells were not stained by $Pt_5(MAA)_8$. These results

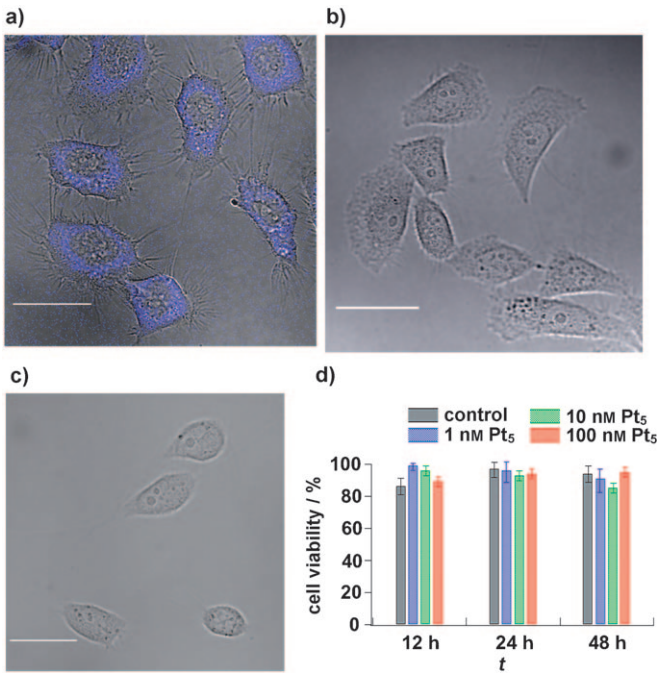


Figure 3. Confocal fluorescence microscopic images merged with differential interference contrast (DIC) images of living HeLa cells labeled with (a) and without (b) $Pt_5(MAA)_8$-(Protein A)-(anti-CXCR4-Ab). c) Confocal fluorescence microscopic images merged with DIC images of living CHO-K1 cells in the presence of $Pt_5(MAA)_8$-(Protein A)-(anti-CXCR4-Ab). The scale bars are 20 um. d) Cell viability of HeLa cells. Cells were incubated with 1, 10, and 100 nM Pt_5 nanoclusters at 37 °C. Black bars show cell viability of control samples in the absence of Pt_5 nanoclusters.

support the conclusion that $Pt_5(MAA)_8$-(Protein A)-(anti-CXCR4-Ab) specifically bound to the chemokine receptor.

Finally, we examined the cytotoxicity of the Pt_5 nanoclusters in HeLa cells by using a cell counter. Figure 3d shows the viability of cells labeled with three different concentrations of Pt_5 nanoclusters (1, 10, and 100 nM) used in the same manner as that shown in Figure 3a and incubated at 37 °C for different lengths of time (12, 24, and 48 h). After 12 hours incubation, more than 89% of cells were alive. Even after 48 hours incubation, cell viability was still more than 85%. These viabilities were comparable to controls. This finding indicates that Pt_5 nanoclusters are harmless fluorescent probes applicable for long-term imaging of living cells.

In conclusion, we synthesized water-soluble, blue-emitting, and atomically monodispersed Pt_5 nanoclusters that have an 18% quantum yield in water. These Pt_5 nanoclusters were purified from other chemical species using size-exclusion HPLC after ligand exchange with MAA. According to ESI mass spectrometry, the molecular formula of the nanocluster was $Pt_5(MAA)_8$. The emission wavelength and lifetime of the Pt_5 nanoclusters (470 nm, (8.8 ± 0.5) ns) were different from those of oxidized PAMAM (G4-OH) (450 nm, (6.3 ± 0.5) ns, see Figure S1 in the Supporting Information). Furthermore, we successfully labeled chemokine receptors in living HeLa cells with $Pt_5(MAA)_8$ bound to an antibody through a conjugated protein to fluorescently image the cells. This achievement suggests that MAA modification can provide an

external coating for Pt nanoclusters that is ideal for the attachment of further proteins or other biomolecules using standard conjugation chemistry. We further demonstrate that Pt_5 nanoclusters have significant cell viability for long-term cellular imaging. Since Pt_5 nanoclusters are less cytotoxic as well as smaller (see Figure S2 in the Supporting Information) than other nanoparticles such as quantum dots[19] and carbon nanotubes,[20] our fluorescent Pt_5 nanoclusters promise to be a useful fluorescent probe for bioimaging and subcellular targeting. Finally, we remark on the fluorescent-wavelength tunability of our Pt nanoclusters. In other nanoclusters, tunability could be achieved by changing the number of atoms in the nanocluster.[1a,b,2,3a,4a,5a] We are trying to do the same in Pt nanoclusters by tuning fluorescence to longer wavelengths such as near infrared (NIR), which is often preferable in bioimaging experiments, as higher wavelengths offer higher penetration depth and lower absorption.

Experimental Section

Materials: Polyamidoamine (G4-OH) (PAMAM (G4-OH)) was purchased from Sigma–Aldrich. H_2PtCl_6, $NaBH_4$, $(NH_4)_2S_2O_8$, *p*-amino benzoic acid, aprotinin, and mercaptoacetic acid (MAA) were purchased from Wako Pure Chemical Industries (Japan). 1-[(3-Dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC) and Protein A were purchased from Thermo Fisher Scientific K.K. *N*-hydroxysulfosuccinimide (Sulfo-NHS) was purchased from Molecular Biosciences Inc. Anti-chemokine receptor (CXCR4) antibody was purchased from BioLegend, Inc. HeLa cells and CHO-K1 cells were purchased from DS Pharma Biomedical Co., Ltd.

Oxidization of PAMAM (G4-OH) with $(NH_4)_2S_2O_8$: PAMAM (G4-OH) (71.4 mg, 0.5 μmol) and $(NH_4)_2S_2O_8$ (0.5 M, 6.0 μL) were added to 2 mL millipore water (18.2 MΩ). This reaction mixture was incubated for two weeks under continuous stirring.[11]

Fluorescence spectra: The excitation and fluorescence spectra of Pt_5 nanoclusters and PAMAM (G4-OH) were measured with a spectrofluorometer (FP-6200, Jasco Inc. Japan). The excitation wavelength for the fluorescence spectra was set to 380 nm for Pt_5 nanoclusters and 375 nm for PAMAM (G4-OH); the emission wavelength for the excitation spectra was set to 470 nm for Pt_5 nanoclusters and 450 nm for PAMAM (G4-OH).

Fluorescence lifetime: A femtosecond pulse from a regenerative amplifier (Libra HE, Coherent) was led to an optical parametric amplifier (OPerA Solo, Coherent), which generated 60 fs pulses at 1 kHz. The beam was directed to two BBO crystals to generate a third harmonic pulse with a wavelength of 380 nm. The excitation light was softly focused into samples contained in 1 cm cuvettes. The power density of the excitation beam was set to approximately 5 $\mu J\,cm^{-2}$, which was sufficiently low to avoid any saturation effects. Time-resolved spectra were obtained using a photon-counting streak camera (C4780, Hamamatsu Photonics, Japan) through a 25 cm monochromator (250is, Chromex).

Size-exclusion high performance liquid chromatography (HPLC) of Pt_5 nanoclusters: We purified Pt_5 nanoclusters from other chemical species using size-exclusion HPLC. To replace PAMAM (G4-OH) ligands with MAA ligands on the Pt nanoclusters, MAA (1 M, 18 μL) was added to Pt nanoclusters samples (300 μL) that included PAMAM (G4-OH) (3 mM). This exchange was feasible because the SH group of MAA has a higher affinity for platinum than PAMAM (G4-OH) nitrogen atoms. The reaction mixture was allowed to stand at room temperature for 30 min. The PAMAM (G4-OH)/MAA molar ratio was set to 1:20. The HPLC system consisted of a 500 μL sample loop, pump (L-2130), UV/Vis absorbance detector (L-2400), and fluorescence detector (L-2485) (Hitachi High-Technologies Corp. Japan). Two TSK gel $G2000SW_{xl}$ size-exclusion HPLC columns (double column) and TSK SW_{XL} guard column were purchased from the TOSOH corp. (Japan). HPLC was performed at room temperature. Phosphate buffer (20 mM, pH 7.5) was used as the mobile phase. The flow rate was maintained at 0.5 $mL\,min^{-1}$ and an injection volume of 100 μL was used for all samples. The detection wavelength for UV absorption was set to 290 nm. The monitoring fluorescence wavelength was 470 nm, and the excitation wavelength was set to 380 nm. Dickson and co-workers have reported the absorption peak of PAMAM (G4-OH) to be around 290 nm.[1c] However, Pt nanoparticles have a much broader absorption band,[21] between 200 and 300 nm, as does MAA.

Inductively coupled plasma mass spectrometry: ICP mass spectra on HPLC fractions from Pt nanocluster samples were measured with an Agilent 7500s instrument (Agilent Technologies, Inc. Headquarters).

Electronspray ionization mass spectrometry: ESI mass spectra of Pt_5 nanoclusters were measured using LTQ XL (Thermo Fisher Scientific K.K.).

Conjugation of Pt_5 nanoclusters with anti-CXCR4-Ab: $Pt_5(MAA)_8$ was conjugated with Protein A using an EDC/Sulfo-NHS coupling reaction. To maintain antibody activity, we used protein A as an adapter protein for the antibody. Protein A can bind specifically to the Fc region of the antibody without blocking the antigen binding site.[16] $Pt_5(MAA)_8$ (57 nM, 400 μL) was activated with EDC (4.56 μL, 0.1 mM) and sulfo-NHS (4.56 μL, 0.1 mM) for 30 min at room temperature. The molar ratio of $Pt_5(MAA)_8$/EDC/NHS was set to 1:20:20. Protein A (24 μM, 9.5 μL) was added to activated $Pt_5(MAA)_8$ and allowed to react for 2 h at room temperature. Finally, $Pt_5(MAA)_8$-(Protein A) was incubated with anti-chemokine receptor (CXCR4) antibody (anti-CXCR4-Ab; 6.67 μM, 13.7 μL) at 4 °C overnight. The concentration of $Pt_5(MAA)_8$ was evaluated using fluorescence correlation spectroscopy with Rhodamine 6G as the standard solution.[22]

Confocal fluorescence microscopy: A culture dish containing HeLa cells was washed with PBS buffer. Dulbecco's modified Eagle's medium (DMEM, 500 μL) was added. Then, $Pt_5(MAA)_8$-(Protein A)-(anti-CXCR4-Ab) (57 nM, 150 μL) was introduced into the dish, which was incubated for 5 min at 37 °C. Confocal fluorescence imaging was performed with an FV1000 instrument (Olympus) using an oil immersion objective lens (60×, N.A. = 1.35) and DAPI (4′,6-diamidino-2-phenylindole) filter. A 405 nm excitation laser was used for fluorescence imaging.

Cell viability tests: Culture medium (5.0 μL) containing HeLa cells was mixed with Trypan Blue (Invitrogen Japan K.K., 5.0 μL). The sample (10 μL) was put on a chamber slide set to a Countess Automated Cell Counter (Invitrogen Japan K.K.) to check for cell viability.

Dynamic light scattering: DLS of Pt_5 nanoclusters in water and ZnSe quantum dots in chloroform were measured using a 633 nm He/Ne laser (Zetasizer Nano, Malvern Instruments Ltd). ZnSe quantum dots were prepared as described.[14] The DLS data show a diameter of (1.3 ± 0.5) nm for Pt_5 nanoclusters, which is much smaller than that of ZeSe quantum dots (7 nm).

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[1] a) J. Zheng, C. Zhang, R. M. Dickson, *Phys. Rev. Lett.* **2004**, *93*, 077402; b) J. Zheng, P. R. Nicovich, R. M. Dickson, *Annu. Rev. Phys. Chem.* **2007**, *58*, 409–431; c) J. Zheng, J. T. Petty, R. M. Dickson, *J. Am. Chem. Soc.* **2003**, *125*, 7780–7781.

[2] Recently, Schaeffer et al. reported that the quantum yield of polymer-stabilized fluorescent gold clusters is 3% in aqueous solution: N. Schaeffer, B. Tan, C. Dickinson, M. J. Rosseinsky, A. Laromaine, D. W. McComb, M. M. Stevens, Y. Wang, L. Petit, C. Barentin, D. G. Spiller, A. I. Cooper, R. Lévy, *Chem. Commun.* **2008**, 3986 – 3988.

[3] a) H. Duan, S. Nie, *J. Am. Chem. Soc.* **2007**, *129*, 2412 – 2413; b) M. Sakamoto, T. Tachikawa, M. Fujitsuka, T. Majima, *J. Am. Chem. Soc.* **2009**, *131*, 6 – 7; c) J. Xie, Y. Zheng, J. Y. Ying, *J. Am. Chem. Soc.* **2009**, *131*, 888 – 889; d) H. Kawasaki, H. Yamamoto, H. Fujimori, R. Arakawa, Y. Iwasaki, M. Inada, *Langmuir* **2010**, *26*, 5926 – 5933; e) T. P. Bigioni, R. L. Whetten, Ö. Dag, *J. Phys. Chem. B* **2000**, *104*, 6983 – 6986; f) T. Huang, R. W. Murray, *J. Phys. Chem. B* **2001**, *105*, 12498 – 12502; g) S. Link, A. Beeby, S. FitzGerald, M. A. El-Sayed, T. G. Schaaff, R. L. Whetten, *J. Phys. Chem. B* **2002**, *106*, 3410 – 3415.

[4] a) C. I. Richards, S. Choi, J.-C. Hsiang, Y. Antoku, T. Vosch, A. Bongiorno, Y.-L. Tzeng, R. M. Dickson, *J. Am. Chem. Soc.* **2008**, *130*, 5038 – 5039; b) J. Zheng, R. M. Dickson, *J. Am. Chem. Soc.* **2002**, *124*, 13982 – 13983; c) T. Vosch, Y. Antoku, J.-C. Hsiang, C. I. Richards, J. I. Gonzalez, R. M. Dickson, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 12616 – 12621.

[5] a) I. Díez, M. Pusa, S. Kulmala, H. Jiang, A. Walther, A. S. Goldmann, A. H. E. Müller, O. Ikkala, R. H. A. Ras, *Angew. Chem.* **2009**, *121*, 2156 – 2159; *Angew. Chem. Int. Ed.* **2009**, *48*, 2122 – 2125; b) L. Shang, S. Dong, *Chem. Commun.* **2008**, 1088 – 1090; c) W. Guo, J. Yuan, Q. Dong, E. Wang, *J. Am. Chem. Soc.* **2010**, *132*, 932 – 934; d) J. Sharma, H.-C. Yeh, H. Yoo, J. H. Werner, J. S. Martinez, *Chem. Commun.* **2010**, *46*, 3280 – 3282; e) H. Xu, K. S. Suslick, *Adv. Mater.* **2010**, *22*, 1078 – 1082; f) E. G. Gwinn, P. O'Neill, A. J. Guerrero, D. Bouwmeester, D. K. Fygenson, *Adv. Mater.* **2008**, *20*, 279 – 283; g) T. Udaya Bhaskara Rao, T. Pradeep, *Angew. Chem.* **2010**, *122*, 4017 – 4021; *Angew. Chem. Int. Ed.* **2010**, *49*, 3925 – 3929; h) Z. Wu, E. Lanni, W. Chen, M. E. Bier, D. Ly, R. Jin, *J. Am. Chem. Soc.* **2009**, *131*, 16672 – 16674.

[6] H. Kawasaki, H. Yamamoto, H. Fujimori, R. Arakawa, M. Inada, Y. Iwasaki, *Chem. Commun.* **2010**, *46*, 3759 – 3761.

[7] a) J. Yu, S. Choi, R. M. Dickson, *Angew. Chem.* **2009**, *121*, 324 – 326; *Angew. Chem. Int. Ed.* **2009**, *48*, 318 – 320; b) J. Yu, S. A. Patel, R. M. Dickson, *Angew. Chem.* **2007**, *119*, 2074 – 2076; *Angew. Chem. Int. Ed.* **2007**, *46*, 2028 – 2030; c) C. I. Richards, J.-C. Hsiang, D. Senapati, S. Patel, J. Yu, T. Vosch, R. M. Dickson, *J. Am. Chem. Soc.* **2009**, *131*, 4619 – 4621.

[8] a) S.-Y. Lin, N.-T. Chen, S.-P. Sum, L.-W. Lo, C.-S. Yang, *Chem. Commun.* **2008**, 4762 – 4764; b) Y.-C. Jao, M.-K. Chen, S.-Y. Lin, *Chem. Commun.* **2010**, *46*, 2626 – 2628; c) C.-A. J. Lin, T.-Y. Yang, C.-H. Lee, S. H. Huang, R. A. Sperling, M. Zanella, J. K. Li, J.-L. Shen, H.-H. Wang, H.-I. Yeh, W. J. Parak, W. H. Chang, *ACS Nano* **2009**, *3*, 395 – 401; d) W. Guo, J. Yuan, E. Wang, *Chem. Commun.* **2009**, 3395 – 3397; e) Y.-C. Shiang, C.-C. Huang, H.-T. Chang, *Chem. Commun.* **2009**, 3437 – 3439; f) J. Xie, Y. Zheng, J. Y. Ying, *Chem. Commun.* **2010**, *46*, 961 – 963; g) G.-Y. Lan, C.-C. Huang, H.-T. Chang, *Chem. Commun.* **2010**, *46*, 1257 – 1259; h) C.-C. Huang, Z. Yang, K.-H. Lee, H.-T. Chang, *Angew. Chem.* **2007**, *119*, 6948 – 6952; *Angew. Chem. Int. Ed.* **2007**, *46*, 6824 – 6828.

[9] J. H. Kim, H. J. Woo, C. K. Kim, C. S. Yoon, *Nanotechnology* **2009**, *20*, 235306.

[10] a) R. M. Crooks, M. Zhao, L. Sun, V. Chechik, L. K. Yeung, *Acc. Chem. Res.* **2001**, *34*, 181 – 190; b) Y. Niu, R. M. Crooks, *Chem. Mater.* **2003**, *15*, 3463 – 3467.

[11] W. I. Lee, Y. Bae, A. J. Bard, *J. Am. Chem. Soc.* **2004**, *126*, 8358 – 8359.

[12] We did not observe any fluorescence from mercaptoacetic acid with 380 nm excitation.

[13] P. Sedláková, J. Svobodová, I. Mikšík, H. Tomás, *J. Chromatogr. B* **2006**, *841*, 135 – 139.

[14] ZnSe qauntum dots were synthesized by the method described in: H.-S. Chen, B. Lo, J.-Y. Hwang, G.-Y. Chang, C.-M. Chen, S.-J. Tasi, S.-J. J. Wang, *J. Phys. Chem. B* **2004**, *108*, 17119 – 17123.

[15] Au_8 nanocluster was synthesized by the method described in Ref. [1c].

[16] Z. D. Liu, S. F. Chen, C. Z. Huang, S. J. Zhen, Q. G. Liao, *Anal. Chim. Acta* **2007**, *599*, 279 – 286.

[17] A. Müller, B. Homey, H. Soto, N. Ge, D. Catron, M. E. Buchanan, T. McClanahan, E. Murphy, W. Yuan, S. N. Wagner, J. L. Barrera, A. Mohar, E. Verástegui, A. Zlotnik, *Nature* **2001**, *410*, 50 – 56.

[18] A. Amara, O. Lorthioir, A. Valenzuela, A. Magerus, M. Thelen, M. Montes, J.-L. Virelizier, M. Delepierre, F. Baleux, H. Lortat-Jacob, F. Arenzana-Seisdedos, *J. Biol. Chem.* **1999**, *274*, 23916 – 23925.

[19] C. Kirchner, T. Liedl, S. Kudera, T. Pellegrino, A. M. Javier, H. E. Gaub, S. Stölzle, N. Fertig, W. J. Parak, *Nano Lett.* **2005**, *5*, 331 – 338.

[20] A. Magrez, S. Kasas, V. Salicio, N. Pasquier, J. W. Seo, M. Celio, S. Catsicas, B. Schwaller, L. Forró, *Nano Lett.* **2006**, *6*, 1121 – 1125.

[21] K. Hikosaka, J. Kim, M. Kajita, A. Kanayama, Y. Miyamoto, *Colloids Surf. B* **2008**, *66*, 195 – 200.

[22] T. Jin, F. Fuji, Y. Komai, J. Seki, A. Seiyama, Y. Yoshioka, *Int. J. Mol. Sci.* **2008**, *9*, 2044 – 2061.

