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Dually functionalized dendrimers by temperature-sensitive surface modification and gold nanoparticle loading for biomedical application

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Abstract

Stimuli-sensitive dendrimers of a new type were developed through dual functionalization of polyamidoamine (PAMAM) dendrimers with temperature-sensitive surface modification using propoxy diethylene glycol (PDEG) and Au nanoparticle (AuNP) loading. These AuNP-hybridized dendrimers were prepared by attaching PDEG to amine-terminated PAMAM G5 dendrimer using 4-nitrophenylchloroformate and subsequent loading of AuNPs into the dendrimer interior via reduction of $AuCl_4^-$ ions with $NaBH_4$ in the dendrimer interior. Transmission electron microscopy showed that AuNP with a diameter of about 2 nm was retained in the PDEG-modified dendrimers. These AuNP-loaded dendrimers were dissolved in aqueous solutions at low temperature but became water-insoluble above a specific temperature, because of the character change of the dendrimer surface from hydrophilic to hydrophobic depending on temperature. The AuNPs in the dendrimer interior generated heat under irradiation of green laser and hence, their association could be induced in the specific area irradiated with green laser. In addition, the combination of the temperature increase and light irradiation was shown to control their interaction with cells, enabling selective cell killing by the AuNP-hybridized temperature-sensitive dendrimers.

Introduction

Dendrimers are a family of synthetic polymers with a regularly branched tree-like structure.[1-4] Such a highly branched backbone gives dendrimers a globular shape that provides both a surface with various properties and an interior capable of encapsulating guest materials. Furthermore, because molecular chains of dendrimers are grown stepwise by repeatedly introducing branch structures to their chain ends, their molecular weights and structures can be controlled precisely. Uniformity in the molecular level and high controllability of the molecular structure and properties make dendrimers highly attractive as a base material for the production of nanomaterials.[5-9]

Dendrimers must be given various functions to increase their usefulness. Therefore, their functionalization has been attempted according to various approaches. Based on the unique structural feature of dendrimers, the surface region of dendrimers presents particularly attractive moieties for their functionalization.[10,11] In fact, various functional moieties such as photosensitive azobenzene[12-14] and *o*-nitrobenzene[15] residues have been incorporated onto the chain terminal of dendrimers to provide photo-responsive functions to dendrimers. Similarly, thermosensitive poly(*N*-isopropylacrylamide)[16-19] chains and their side chain moieties, such as *N*-isopropylamide and *N*-isobutyramide

groups,[20-22] have also been conjugated to dendrimer chain terminals to provide temperature-sensitive properties.

The interior of dendrimers can be another site for giving functions to dendrimers. Small molecules[23-28] and nano-sized particles[29-32] of various kinds were shown to be encapsulated in the dendrimer interior. Therefore, various functions can be provided to dendrimers via encapsulation of guest materials with various functionalities. Furthermore, different functions can be provided to dendrimers using surface and interior moieties separately. The combination and synchronization of these functions can generate new and higher-order functions of the dendrimers. Dendrimer functions can raise their importance as nanomaterials for applications in fields such as biomedicine and drug delivery.[33-35]

In this study, we attempted to use a new strategy for the production of highly functional dendrimers. We engineered PAMAM dendrimers dually through modification of the surface moiety with temperature-sensitive groups and loading with photosensitive gold nanoparticles (AuNPs), which exhibit a unique photo-induced heat-generating ability derived from surface plasmon resonance (SPR)[36-39], in the interior (Fig. 1). Higher-order functions of the dually functionalized dendrimers, such as the controls between hydrophilic and hydrophobic character change and between dissolution and

precipitation change, in temperature-responsive, photo-responsive and their synchronous fashions were described.

Experimental

Materials. Propoxy di(ethylene glycol) (PDEG), Gold(III) chloride hydrate ($HAuCl_4 \cdot 4H_2O$), sodium borohydride ($NaBH_4$) and 3-(4,5-dimethyl-2-thiazoryl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) were obtained from Wako Pure Chemical Industries Ltd. (Osaka Japan). 4-Nitrophenyl chloroformate, PAMAM dendrimers of the fifth generation (G5), propidium iodide (PI), and Dublecco's modified Eagle's medium were obtained from Sigma-Aldrich Japan (Tokyo, Japan). Calcein-acetoxymethyl ester (calcein-AM) was from Nacalai Tesque (Kyoto, Japan). Tetrahydrofuran (THF), dimethyl sulfoxide (DMSO) and triethylamine (TEA) were supplied from Kishida Chemical (Osaka, Japan). Tetrahydrofuran (THF) and dimethyl sulfoxide were distilled just prior to use.

Synthesis of AuNP-hybridized dendrimers. Propoxy diethylene glycol 4-nitrophenyl carbonate (PDEG-NPC) was synthesized by the reaction of PDEG with 4-nitrophenyl chloroformate according to our previous paper (see Figure S1 in supporting information).[40] PDEG-modified PAMAM G5 (PDEG-G5) dendrimer was

synthesized and characterized according to our previous report (see Figures S2 and S3 in supporting information).[40] Hybridization of AuNP into dendrimers was performed according to our previous reports.[30,41] In brief, an aqueous solution of PDEG-modified dendrimer (74.6 mg, 146 μM, 10 ml) and 10 or 55 equivalent of aqueous solution of $HAuCl_4$ (10 mM, 1.46 ml or 8.03 ml) was mixed. The mixed solution was reduced by adding a 5-fold molar excess of $NaBH_4$ (150 mM) dissolved in 0.3 M NaOH (487 μl or 2.68 ml) along with vigorous stirring for 1 h. Then the AuNP-loaded dendrimer was purified by dialysis against distilled water. AuNP moiety of the AuNP-hybridized dendrimers were analyzed with transmission electron microscopy (TEM) using a JEOL2000 with carbon-coated copper grids.

Quantification of Au atoms in dendrimer. AuNP-loaded dendrimer samples were dried under vacuum in the presence of P_2O_5 overnight. The fully dried dendrimer samples were first carefully weighted, and then dissolved in freshly prepared HCl/HNO_3 (aqua regia). These sample solutions were then diluted with distilled water to 0.5 mg/mL, and the Au concentration was measured with inductively coupled plasma mass spectroscopy (SPS7800, Seiko Instruments Inc., Japan).

Turbidity measurements. Turbidity of temperature-sensitive dendrimers in 50 mM phosphate solutions (pH 7.0, 1.5 mg/mL) was measured at 700 nm using a Jasco

Model V-560 spectrophotometer equipped with a Peltier type thermostatic cell holder coupled with a controller ETC-505T. The heating rate of sample cells was adjusted at 1.0 °C min^{-1}. The cloud point was taken as the initial break points in the resulting transmittance versus temperature curves. For estimation of temperature-induced reversible aggregation and dissociation of dendrimers, temperature of cuvette folder in the Jasco Model V-560 spectrophotometer was changed between 15 ^{o}C and 35 ^{o}C alternatingly every 3 min.

Recovery by centrifugation. Dendrimers in 50 mM phosphate solutions (pH 7.0, 1.5 mg/mL, 3 mL) were incubated at 60 ^{o}C for 10 min and then were centrifuged (15000 rpm) for 20 min at 40 ^{o}C. Absorption spectra of the supernatant was measured using a Jasco Model V-560 spectrophotometer equipped with a Peltier type thermostatic cell holder coupled with a controller ETC-505T. The precipitate was again dissolved in 50 mM phosphate solution (pH 7.0, 3 mL) and its absorption spectra were measured.

Analytical TEM observation. Atomic-resolution HRTEM and STEM observations were performed with a 200 kV field-emission transmission electron microscope (JEM-2100F, JEOL, Japan) equipped with a scanning unit and an energy dispersive X-ray spectroscopy (EDS) detector. To prepare stained dendrimers for EDS, the dendrimer solution was deposited on a carbon-coated copper grid, and then excess

of dendrimer solution was removed with a filter paper. Then, a drop of aqueous sodium phosphotungstate solution (2%, pH7.4) was again deposited on the grid and removed with a filter paper.[42] The EDS line-scan analyses were performed using JEOL JED-2310S analysis system. The specimen drift-correction programs are used for the EDS line-scan analysis.[43]

Cell culture. HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO), supplemented with 10% fetal bovine serum (FBS, GIBCO), 50 units/mL penicillin, and 50 μg/mL streptomycin at 37 ^{o}C under 5% CO_2 condition.

Photo-irradiation experiment. The solution of AuNP-loaded dendrimers of a given concentration of dendrimer (3 ml) was irradiated by the Nd:YVO_4 LASER (JUNO1000, λ=532 nm) at 12 W/cm^2. The sample temperature was monitored by SK-1250MC (Sato Keiryoki MFG. Co., Ltd., Japan) immersed in the solution of the cell. The initial temperature of the solution before the light irradiation was 24 ^{o}C.

Photocytotoxicity. HeLa cells (2×10^5 cells) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) for 2 days in 35-mm dishes, and were washed with phosphate-buffered saline (PBS; 8 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 3 mM KCl, 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$). The AuNP-loaded dendrimers dissolved in the phenol red-free Opti-MEM were added to the

HeLa cells and incubated at 37 ^{o}C for 2h. Then, the cells were washed with PBS and immersed in fresh Opti-MEM (2 ml). The cells around the central region of the 3 mm diameter dish were irradiated with light (λ=532 nm) using Nd:YVO_4 SHG LASER (JUNO10000) at ca. 12 W/cm^2 for 15 min and further incubated for 3 h at 37 ^{o}C. The staining solution was prepared by adding 10 μL of calcein-AM in DMSO solution (1 mg/ml) and 15 μL of aqueous PI solution (1 mg/ml) to 15 ml of PBS. The staining solution (2 ml) was added to the cells and incubated for 30 min at 37 ^{o}C. Then, the cells were washed with PBS, and observed with a fluorescence microscope (IMT-2, Olympus, Japan).

Results and discussion

Temperature-responsive properties of AuNP-loaded dendrimers

Dually functionalized dendrimers were prepared according to the procedure shown in Figure 2A. The amine-terminated PAMAM G5 dendrimers were first reacted with PDEG-NPC to modify their surface with thermosensitive PDEG groups as reported previously (see supporting information).[40] Then, AuNPs were introduced by reducing $AuCl_4^-$ ions in the PDEG-modified dendrimers. Previous studies have shown that uniform AuNPs with 2-4 nm diameters were obtainable by loading of $HAuCl_4$ in the PAMAM dendrimers and subsequent reduction with an appropriate reducing agent such

as $NaBH_4$.[31,44] We have already achieved AuNP-loading into poly(ethylene glycol)-modified PAMAM dendrimers using the same method.[30,41] AuNP-loaded dendrimers of two kinds were prepared by adding 10 and 55 equivalents $HAuCl_4$ per dendrimer and reducing it in the dendrimer subsequently. Generation of AuNP in the PDEG dendrimers was confirmed from their extinction spectra exhibiting a broad peak around 530 nm, which is typical for those of AuNP (Fig. 2B).[31,44] These AuNP-loaded dendrimers are designated, respectively, as PDEG-G5-Au_{10} and PDEG-G5-Au_{55}. Au contents of these AuNP-loaded dendrimers were quantified with inductively coupled plasma mass spectroscopy and numbers of Au atoms per dendrimer were estimated to be 9.0 and 53.3 for PDEG-G5-Au_{10} and PDEG-G5-Au_{55} respectively, indicating that Au ions added to dendrimers were quantitatively converted to AuNPs in the dendrimer. This result agrees well with recent findings that the precise synthesis of inorganic nanoparticles or subnanoparticles with a controlled number of metal atoms have been achieved using a dendrimer-type template.[45-47] We also prepared AuNP-loaded dendrimers using PAMAM G5 with OH terminal groups, which were designated as OH-G5-Au_{10} and OH-G5-Au_{55}, as temperature-insensitive controls.

First, we examined the influence of AuNP-loading on temperature-sensitive property of the dendrimer by detecting the cloud point of their solutions (Fig. 3). As

presented in Figure 3A, PDEG-G5 dendrimer exhibited a cloud point around 25°C, indicating that this dendrimer lost water solubility around this temperature. We have shown that water solubility of temperature-sensitive PAMAM dendrimers was controlled by characters of surface of the dendrimers, where temperature-sensitive moieties, such as *N*-alkylamide groups and oligo ethylene glycol groups, are densely accumulated.[20-22,40] These groups in the dendrimer periphery are well hydrated at low temperature. However, as temperature rises, dehydration of these groups occurs at a specific temperature, resulting in phase transition of these dendrimers from hydrophilic to hydrophobic.[21,22,40] Indeed, AuNP-loaded dendrimers exhibited a cloud point, suggesting that their water-solubility might be controlled by hydration and dehydration of their surface moiety. However, the fact that PDEG-G5-Au_{10} and PDEG-G5-Au_{55} showed cloud points around 28°C and 30°C, respectively, indicates that loading of AuNP caused elevation of the cloud point of the dendrimer. Association of AuNP with tertiary amines and amide groups included in the dendrimer backbone through coordinate bonding may enhance their polarities and increase the hydrophilicity of the dendrimer interior, resulting in increase of the cloud point. Their aggregation and dissolution were shown to be repeated responding to the ambient temperature below and above the cloud point (Fig. 3B), indicating that dehydration and rehydration of the

dendrimer surface took place repeatedly. Time lags exist between temperature change and their response because it took a few minutes to reach the determined temperature in the dendrimer solution after adjusting the temperature of the cuvette folder (Fig. 3C).

Because aggregation and dissociation of the AuNP-loaded PDEG-modified dendrimers were controlled by ambient temperature, we examined recovery of these dendrimers by incubating the AuNP-loaded dendrimers above cloud point (60°C) for 10 min and precipitating them using centrifugation at 15000 rpm for 20 min at 40°C (Fig. 4A). Absorption spectra for the dendrimer solution before centrifugation and supernatant after centrifugation were shown in Fig. 4B. Comparison between absorbance at 530 nm which is derived from SPR of AuNPs indicates that around 93% of the Au-loaded dendrimers were precipitated by this process. The precipitate was again dissolved in the same volume of the buffer below the cloud point. The solution showed a spectrum similar to the original solution, indicating that the precipitated AuNP-loaded dendrimers were efficiently dissolved in the buffer at low temperature and about 83% of the AuNP-loaded dendrimer was recovered through precipitation and subsequent re-dissolution as judged from absorbance at 530 nm for the spectra, even though precipitation was made under strong centrifugal force (Fig. 4B). In the same manner, efficiencies of recovery for various kinds of AuNP-loaded dendrimers with

centrifugation and subsequent re-dissolution were estimated and the result was shown in Fig. 4C. The AuNP-loaded dendrimers loaded with temperature-sensitive property showed efficient precipitation and re-dissolution, but the temperature-insensitive OH-terminated dendrimers were not precipitated by centrifugation. The result indicates that AuNPs were retained tightly in the interior of the dendrimers and their aggregation and dissolution were controlled by the surface property change of the dendrimers responding to ambient temperature.

Characterization of AuNP-loaded dendrimers with TEM

We further investigated temperature-controlled aggregation and dissolution of AuNP-loaded dendrimers using TEM (Fig. 5). The temperature of the AuNP-loaded dendrimer solutions was increased from 25°C to 60°C and was maintained for 10 min. Subsequently, the solution temperature was returned to 25°C and kept for 1 min. The TEM image of PDEG-G5-Au_{10} displayed individual AuNPs with 2 nm diameter, suggesting that AuNPs were well stabilized by the dendrimers before heating. However, after heating, particles with much larger sizes and with protean shapes were observed, demonstrating the occurrence of aggregation of AuNPs. After the returning the temperature to 25°C, individual AuNPs were again observed, showing that dissociation of the aggregate was induced quickly at this temperature. Indeed, such

temperature-induced aggregation was not observed for OH-G5-Au_{10}, which is a temperature-insensitive dendrimer. Considering that the cloud point of PDEG-G5-Au_{10} dendrimer was around 28°C (Fig. 3A), this result again indicates that aggregation and dissociation of AuNPs were controlled by the surface property of the temperature-sensitive dendrimers encapsulating them and that they were tightly retained in their interior during their association and dissociation responding to the temperature change. The temperature-induced reversible association and dissociation for the AuNP-hybridized dendrimers was further confirmed by the size estimation of AuNPs based on TEM observation. The mean diameters of AuNP moieties for PDEG-G5-Au_{10} and PDEG-G5-Au_{55} dendrimers were estimated as 2.2 +/- 0.9 nm and 1.9 +/- 0.6 nm, respectively, before heating and 2.3 +/- 0.8 nm and 2.0 +/- 0.6 nm, respectively, after heating at 60°C for 10 min and subsequent cooling at 25°C for 1 min.

In spite of difference in amount of Au atoms loaded into the dendrimers, PDEG-G5-Au_{10} and PDEG-G5-Au_{55} showed similar sizes of AuNPs. We observed PDEG-G5-Au_{10} using a high resolution TEM and confirmed that AuNPs with a diameter less than 1 nm hardly appeared (Fig. S4). Probably, generation and growth of AuNPs might not take place homogeneously in all dendrimer molecules during the AuNP production in the dendrimer. We observed that PDEG-G5-Au_{55} exhibited a cloud

point at higher temperature than PDEG-G5-Au_{10} (Fig. 2A). A possible explanation may be difference in fraction of the dendrimers containing AuNPs. In fact, we observed that mixtures of PDEG-G5-Au_{55} and empty PDEG-G5 dendrimers dissolved in the buffer exhibited cloud points at different temperatures depending on their ratios (Fig. S5).

We attempted to prove encapsulation of AuNPs by PDEG-G5 dendrimers using analytical scanning transmission electron microscopy (STEM) observation and the line-scan analysis of energy dispersive X-ray spectroscopy (EDS).[43] Figure 6A depicts STEM image for PDEG-G5-Au_{10} stained with phosphotungstate, which is used for staining of PAMAM dendrimers.[42] We performed EDS analysis for two particles indicated as **a** and **b** in Figure 6A. As is shown in Figures 6B and 6C, for each particle, Au-rich area is surrounded by a tungsten (W)-rich line area with about 8 nm, which corresponds to the diameter of PDEG-G5-Au_{10} dendrimer (7.95 nm) estimated with DLS (Fig. S6). Result might indicate that AuNP was located in the interior of the dendrimer.

Light-sensitive properties of AuNP-loaded dendrimers.

The AuNP moiety of the dendrimer can be used as a transducer, which converts light signal to heat signal. Therefore, the AuNP-loaded PDEG-modified dendrimers are expected to exhibit light responsive behaviors. We irradiated the laser light into the dendrimer solutions using a Nd:YVO_4 laser (λ=532 nm) and temperature of the

solutions were monitored (Fig. 7A). As presented in Figures 7B and 7C, temperatures of both PDEG-G5-Au_{10} and PDEG-G5-Au_{55} dendrimer solutions increased significantly with time during laser irradiation. Furthermore, the PDEG-G5-Au_{55} dendrimer solution exhibited more significant temperature increase upon light irradiation. These facts indicate that the AuNP-loaded dendrimers indeed have photo-responsive heat-generating ability and that those with a higher amount of Au atoms have a higher heat-generating capability. Figure 7D depicts images of the PDEG-G5-Au_{55} dendrimer solution during laser light irradiation without stirring. After 30-s irradiation, intensity of laser light scattering increased slightly. Then the light scattering became more intense with time, indicating that dendrimer aggregation proceeded with time under the light irradiation. It is particularly interesting that marked aggregation of dendrimers was induced in the light-passing region, but other portions of the solution remained transparent (Fig. 7D). This result indicates that the surface character of the dendrimer existing at a specific region of the solution can be changed by irradiating that region with the laser (Fig. 7E).

Dual control of AuNP-loaded dendrimers with cells

Considering their possible application to photothermal therapy, we examined temperature-induced and light-induced dual control of cell-killing using the AuNP-loaded dendrimers (Fig. 8). The temperature-sensitive PDEG-G5-Au_{55} dendrimer was added to HeLa cells, a cervical cancer cell line, and incubated at 37 °C for 2 h. After washing and staining with calcein-AM and propidium iodide (PI), which respectively show live and dead cells,[48] the cells only slightly displayed PI fluorescence,

demonstrating that the dendrimer did not induce cellular death under this condition without laser irradiation (Fig. 8A). However, cells with the same treatment were irradiated with the laser (λ=532 nm) for 30 min, we observed considerable PI fluorescence around the laser-irradiated area (Fig. 8B), indicating that the light irradiation induced cellular death efficiently. When the cells were incubated with the same AuNP-loaded dendrimer at 30 °C for 2 h and the laser was irradiated, the cells showed a very low extent of PI fluorescence. Considering that the PDEG-G5-Au_{55} dendrimers changed the character from hydrophilic to hydrophobic at temperatures higher than 30°C (Fig. 3A), it is likely that the dendrimers hardly associate with the cells because of their hydrophilic surface at 30°C, but their association to the cells might be enhanced at 37°C because of the hydrophobic characteristics of the dendrimers (Fig. 8F). In fact, we have observed that cellular association of PDEG- and ethoxy diethylene glycol-modified PAMAM dendrimers was greatly enhanced above the cloud point.[40] Therefore, the AuNPs retained in the dendrimers might generate heat upon laser irradiation, resulting in efficient cellular death only for cells treated with the dendrimers at 37°C. When the temperature-insensitive OH-G5-Au_{55} dendrimers were used for the cellular treatment, such a photo-induced cellular death was not observed irrespective of the incubation temperature (Figs. 8 D,E). This result again reflects that the hydrophobic

surface of the PDEG-G5-Au_{55} dendrimers might be responsible for the efficient photo-induced cell-killing effects at 37°C.

Considering their application to practical therapy, it is necessary to use AuNPs which can be activated by near infrared light that penetrates efficiently into the body.[49] This problem can be solved by incorporating AuNPs with asymmetric shape, such as Au nanorods, in the dendrimer interior or by modifying their shape in the dendrimer.[50,51] Also, incorporation of photosensitizers, which modify absorption property of AuNPs, into the dendrimer interior may be another way to increase their photothermal properties under near infrared light irradiation.[52] Alternatively, stimuli of other kinds might be used to induce heat generation from the AuNPs hybridized with the dendrimers.[53] For example, AuNPs were shown to generate heat under radiofrequency fields.[54] Furthermore, these AuNP-hybridized dendrimers might be advantageous from the viewpoint of their detection of biodistribution because AuNP moiety of the dendrimers might enable highly sensitive detection of the dendrimers by AuNP-based imaging techniques of various kinds including X-ray computed tomography and ultrasound imaging.[55,56]

Conclusions

This study demonstrated that hybridization of photo-responsive AuNPs with the temperature-sensitive dendrimers can generate new functional nanomaterials of which the characters and functions can be controlled dually by ambient temperature and light irradiation. Such dual signal sensitive property of the dendrimers could contribute to

increase efficacy of various kinds of non-invasive tumor therapies including photothermal therapy. Furthermore, combinations of other types of stimuli-sensitive dendrimers and nanoparticles may generate hybrid nanomaterials exhibiting other stimuli-responsive functions. Therefore, our approach might be efficient for the production of nanomaterials with a high order of functions, which can contribute to the generation of new efficient medicines.

Supporting Information

Supporting Information is available from the Royal Society of Chemistry

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Notes

The authors declare no competing financial interest.

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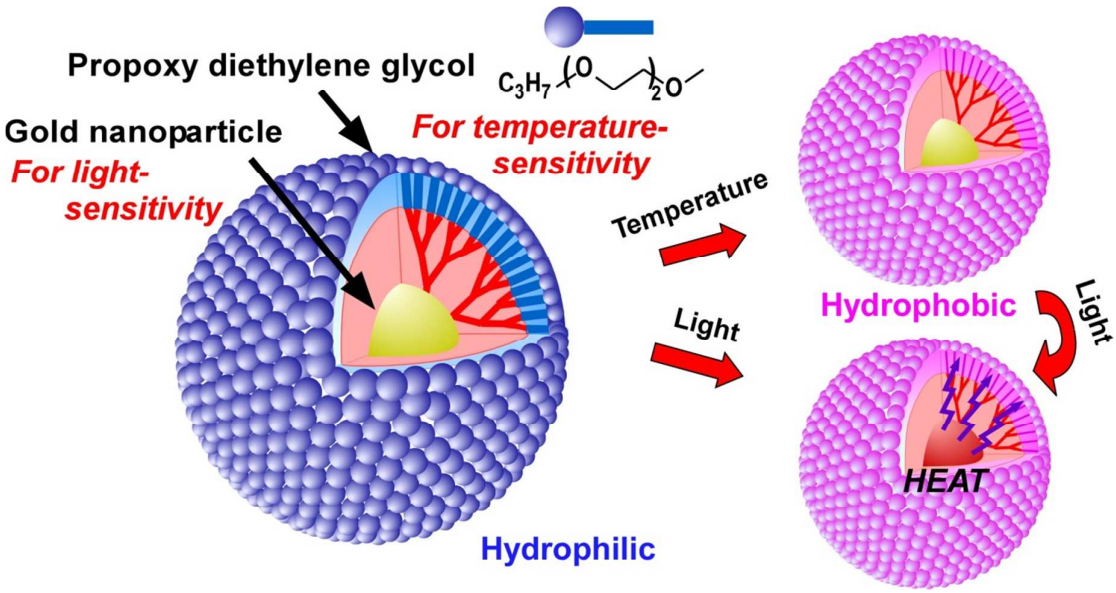

Figure 1. Design of dually functionalized dendrimer for temperature and light controlled functions by hybridization of temperature-sensitive propoxy diethylene glycol-modified dendrimer with light-sensitive gold nanoparticle.

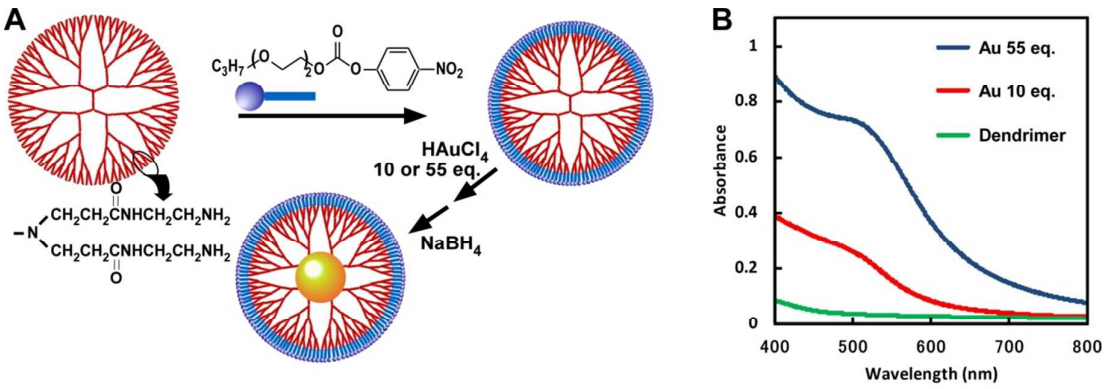

Figure 2. (A) Synthetic route for dually functionalized dendrimer for temperature and light controlled functions by surface modification of amine-terminated polyamidoamine G5 dendrimer with propoxy diethylene glycol for temperature-sensitivity and gold nanoparticle loading for light sensitivity. (B) (C) UV-vis spectra for aqueous solutions of PDEG-G5-Au_{55}, PDEG-G5-Au_{10}, and PDEG-G5 dendrimers. [Dendrimer] = 14.6 μM.

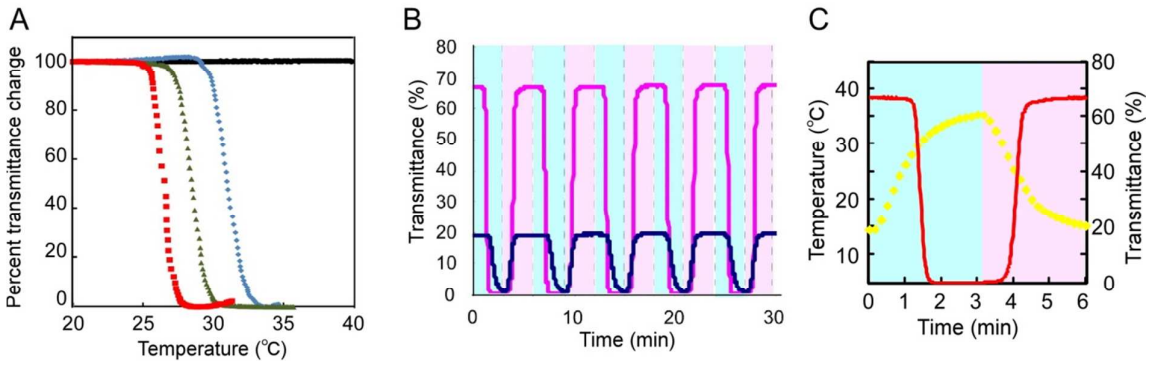

Figure 3. Influence of AuNP-loading on the cloud point of PDEG-G5 dendrimers dissolved in 50 mM phosphate buffer (pH 7.0). [Dendrimer] = 1.5 mg/ml. (A) Temperature-dependence of percent change in transmittance (λ=700 nm) for PDEG-G5 (red line), PDEG-G5-Au_{10} (green line), PDEG-G5-Au_{55} (blue line) and OH-G5-Au10 (black line) solutions. (B) Reversible change of transmittance for PDEG-G5-Au_{10} (red line) and PDEG-G5-Au_{55} (blue line) solutions responding to ambient temperature between 15°C (light blue areas) and 35°C (pink areas). (C) Time courses of temperature (red line) and transmittance (yellow line) of PDEG-G5-Au_{10} solution responding to ambient temperature between 15°C (light blue areas) and 35°C (pink areas).

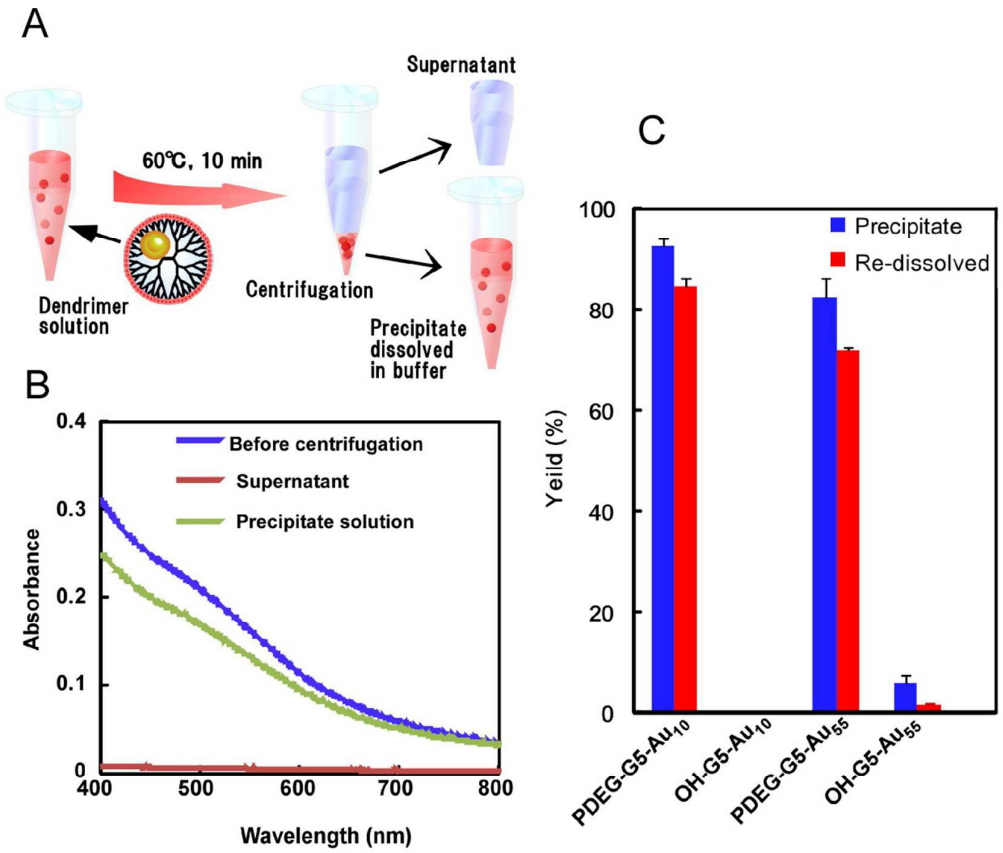

Figure 4. Temperature-induced recovery of dendrimers and re-dissolution of precipitated dendrimers. (A) Illustration of experimental procedure. (B) Absorption spectra of PDEG-G5-Au_{10} (0.6mg/ml) dissolved in 50 mM phosphate (pH7.0) before and after centrifugation and precipitated PDEG-G5-Au_{10} re-dissolved in the same buffer. (C) Percent precipitation and recovery of various AuNP-loaded dendrimers.

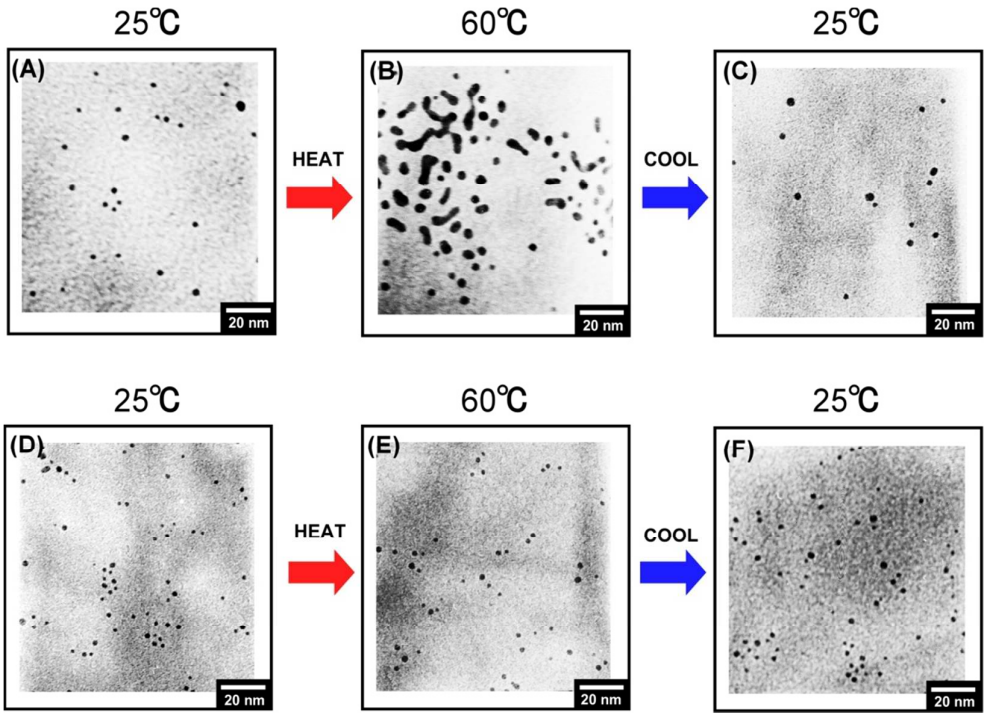

Figure 5. TEM images of PDEG-G5-Au_{10} (A,B,C) and OH-G5-Au_{10} (D,E,F) dendrimers before heating (A,D), after heating at 60°C for 10 min (B,E), and after subsequent cooling for 1 min at 25°C (C,F).

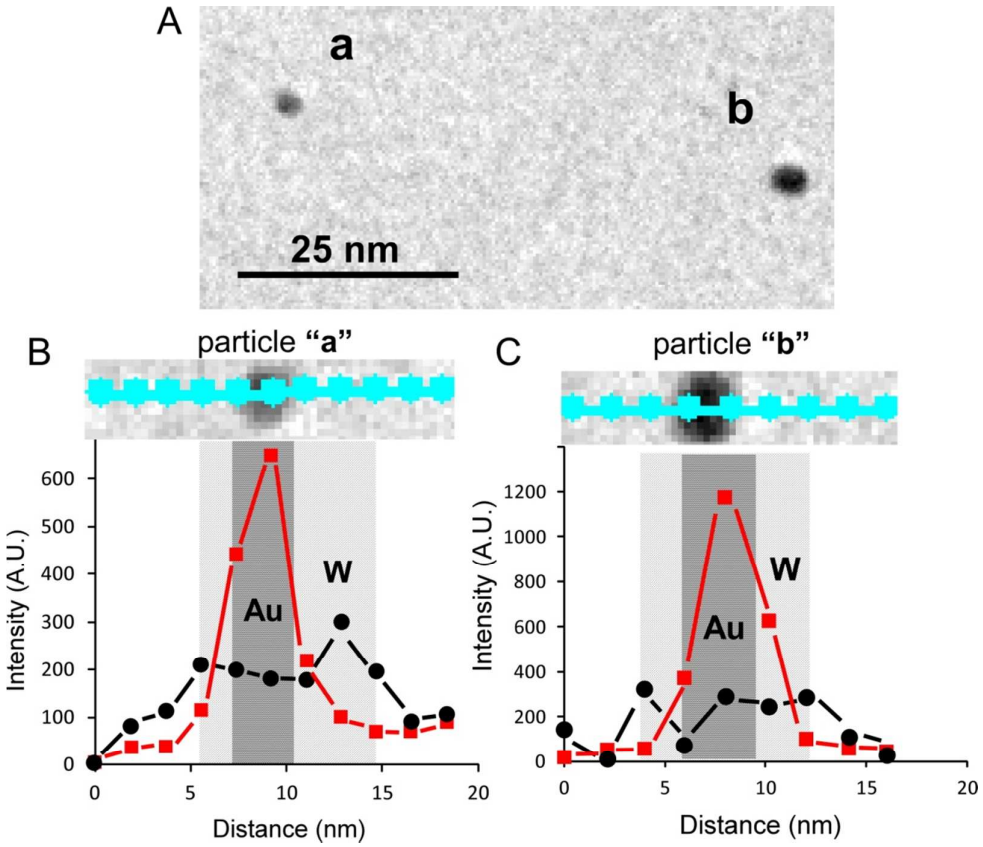

Figure 6. STEM-energy dispersive spectroscopy analysis for PDEG-G5-Au_{10}. STEM image of PDEG-G5-Au_{10} dendrimers (A) and intensity profiles for Au and tungsten (W) along the lines for the particles "a" (B) and "b" (C) shown in A.

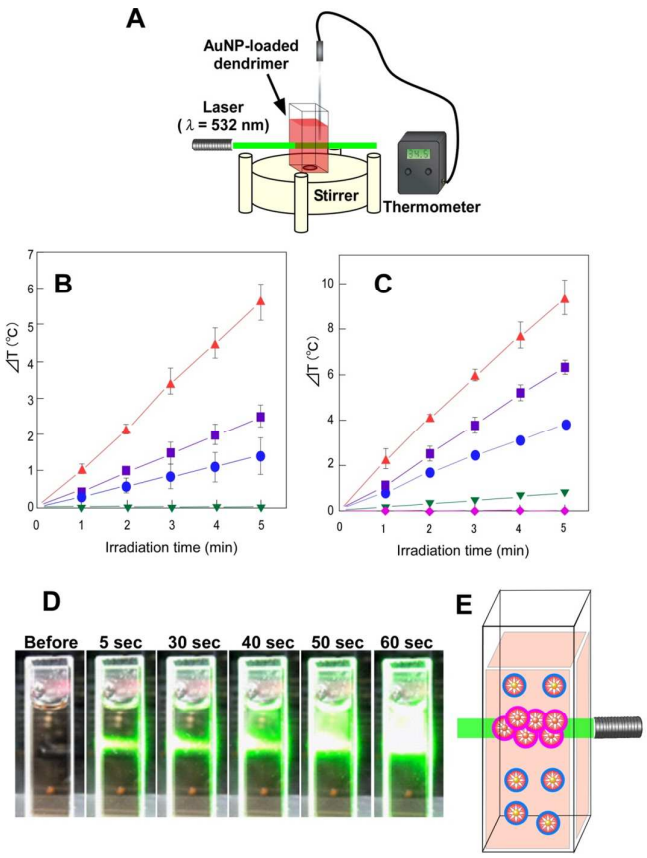

Figure 7. Photoirradiation-induced increase in temperature of AuNP-loaded PDEG-G5 dissolved in 50 mM phosphate-buffered solution (pH 7.0). (A) Apparatus for the measurement. Temperature increase in PDEG-G5-Au_{10} (B) or PDEG-G5-Au_{55} (C) dendrimer solutions during laser irradiation with stirring. Dendrimer concentrations were 0 μM (diamonds), 1 μM (inverted triangles), 6 μM (circles), 15 μM (squares), and 30 μM (triangles). The initial temperature was 24°C. (D) Time-dependent induction of PDEG-G5-Au_{55} dendrimer aggregation duaring laser irradiation for 60 s. (E) Schematic illustration of laser irradiation-induced association of PDEG-G5-Au_{55}. Dendrimer solution (30 μM) was irradiated with laser without stirring.

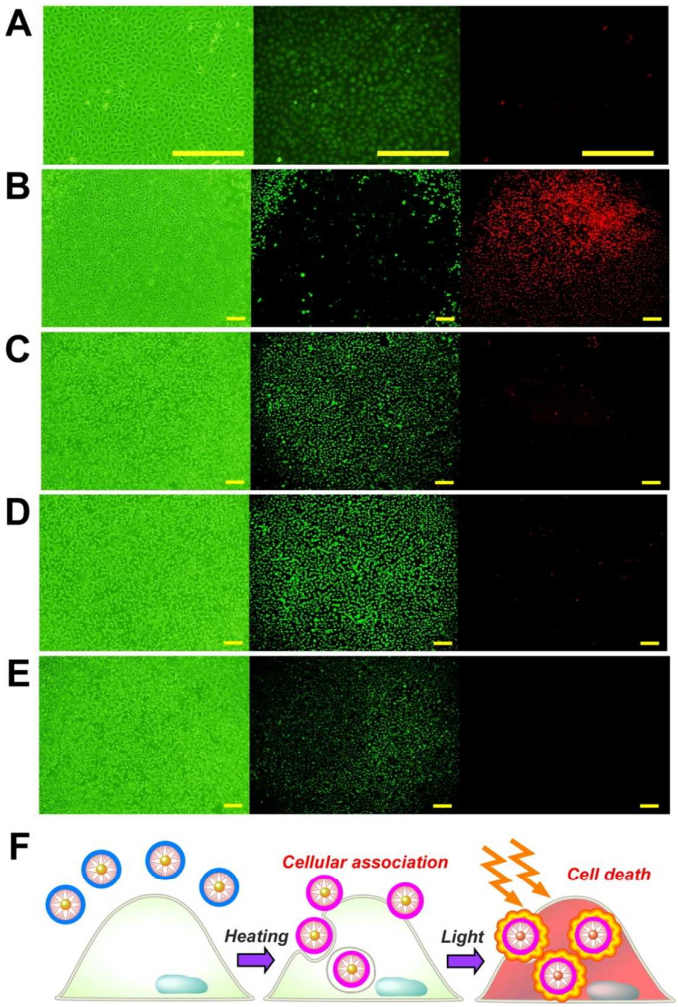

Figure 8. Phase contrast (left), calcein-AM fluorescence (center) and PI fluorescence (right) micrographs for HeLa cells treated with PDEG-G5-Au_{55} (A,B,C) and OH-G5-Au_{55} (D,E)) for 2h at 37°C (A,B,D) or 30°C (C,E) before (A) and after (B,C,D,E) laser irradiation for 30 min. (F) Schematic illustration of temperature- and light irradiation-induced control of cell death by PDEG-G5-Au_{55} dendrimer. [Dendrimer] = 15 μM. Bars represent 200 μm. Calcein-AM fluorescence and PI fluorescence respectively show living and dead cells.