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Gold Nanoparticles Coated with a Thermosensitive Hyperbranched Polyelectrolyte: Towards Smart Temperature and pH Nanosensors

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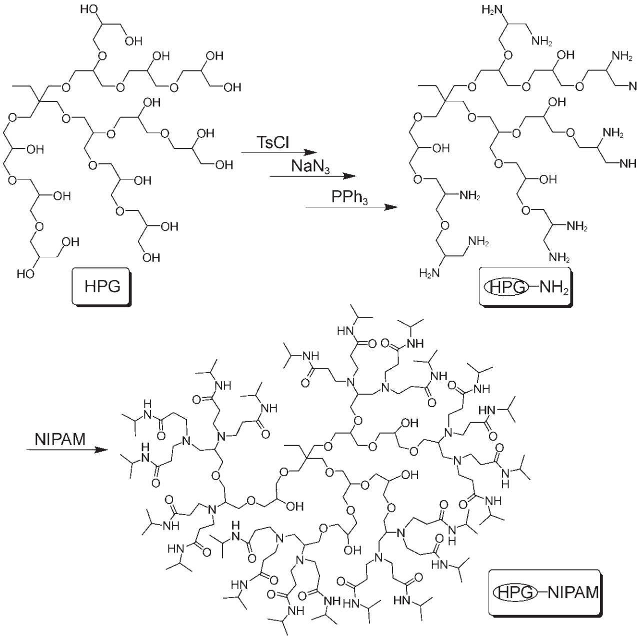
Metal and semiconductor nanoparticles are emerging as a new class of functional building blocks for sensing and biodetecting devices owing to the easily detectable shifts of their unique optical and electronic properties upon alteration of the local environment and aggregation states.[1–4] In this context, a number of gold nanoparticle based colorimetric assays for biomolecules have been developed which take advantage of the drastic red shift of their surface plasmon band induced by the strong interparticle coupling after aggregation.[5–7] Recently, tremendous research efforts have been devoted to designing nanoparticles with surface coatings responsive to external stimuli (temperature and pH)[8–14] or specific analytes.[5–7,15–18] Among them, thermo- and pH-sensitive nanoparticles are of particular interest for sensors and for the controlled release of active payloads, such as low-molecular-weight molecular drugs.[8–14] Herein, we present the development of new thermosensitive gold nanoparticles with tunable lower critical solution temperature (LCST) by coating the nanoparticles with thermo- and pH-responsive hyperbranched polyelectrolytes.

Most established strategies to prepare thermosensitive gold nanoparticles rely on grafting linear thermosensitive polymers with active end groups, for example, thiols, onto the nanoparticle surface.[8–12] In these cases, because the LCST of the linear polymers exhibits little dependence on the structural parameters, such as molecular weight, the transition temperatures of the resulting nanoparticles are directly associated with the polymer of choice and can only be modulated in a narrow temperature range (of less than 5 °C).[19] However, there is an increasing demand for approaches leading to nanoparticles with flexibly tunable transition temperatures, which will better meet the needs of a broader range of applications. Recent advances indicate that the phase transitions of dendritic and hyperbranched thermosensitive polymers show a stronger dependence on their molecular weight, owing to the densely packed thermoresponsive groups in their globular structures.[20–22] The controlled synthesis of hyperbranched polyglycerols (HPGs) is possible through ring-opening multibranching polymerization of glycidol under slow monomer-addition conditions.[23] On the other hand, poly(*N*-isopropyl-acrylamide) (PNIPAM) is a well-known thermosensitive polymer, exhibiting a LCST at around 32 °C.[24] Recently, Kono and co-workers showed that polyamidoamine (PAMAM) dendrimers with *N*-isopropylacrylamide (NIPAM) terminal groups became thermosensitive after surface modification.[21] In the work presented herein, HPGs modified with NIPAM groups, which are thermosensitive, are used to coat water-soluble gold nanoparticles through noncovalent bonds, giving rise to nanoparticles with readily controllable LCSTs in a very broad temperature range (25–55 °C).

HPGs with NIPAM groups (HPG$_n$-NIPAM, where *n* is the degree of polymerization plus three)[23] were synthesized for the first time by a four-step reaction sequence (Scheme 1), namely tosylation with *p*-toluenesulfonyl chloride, substitution reaction with sodium azide, Staudinger reaction with triphenylphosphine, and finally Michael addition with NIPAM monomers.[25] ^{1}H NMR spectroscopy confirmed that all three HPGs used here were successfully modified with



Scheme 1. Synthetic route for NIPAM-terminated hyperbranched polyglycerols. NIPAM = *N*-isopropylacrylamide.

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more than 90 % degree of functionalization. The structural parameters of these three HPG-NIPAM samples and their corresponding precursors are summarized in Table 1.

Table 1: Structure parameters and LCSTs of HPG-NIPAM polymers.

Polymer	M_n of HPG precursor [g mol^{-1}][a]	PDI of HPG precursor[a]	Molecular weight[b] [g mol^{-1}]	LCST [°C][c]
HPG_{18}-NIPAM	1240	1.60	4900	49.2
HPG_{38}-NIPAM	2700	1.95	10400	47.6
HPG_{76}-NIPAM	6600	2.60	22500	33.5

[a] M_n values and polydispersity (PDI) were obtained from size exclusion chromatography (SEC) in DMF. [b] Molecular weights were calculated from ^{1}H NMR spectra. [c] LCSTs were determined at pH 9.0 with a polymer concentration of 2 mg mL^{-1}.

The HPG-NIPAM polymers are highly thermosensitive, as confirmed by the sharp decreases in optical transmittance when their aqueous solutions (2.0 mg mL^{-1}, pH 9.0) are heated to a certain temperature (Figure 1 a,b). The LCST of HPG-NIPAM shows a strong dependence on molecular weight, that is, doubling of molecular weight from 10400 to 22500 led to a decrease of the LCST by about 15 °C. In pronounced contrast, a previous study on linear PNIPAM showed that the LCST dropped by only 2 °C when the average molecular weight was increased from 5400 to 160000.[19] The mechanism of dehydration of PNIPAM above the LCST involves a weakening of the hydrogen bonding between the amide bonds and water molecules at elevated temperature and an increasing role of hydrophobic interactions between the hydrophobic isopropyl groups.[24] In the globular structure of HPG-NIPAM, the higher packing density of NIPAM groups in polymers with elevated molecular weight can lead to more efficient interactions; thus, the phase transition occurs at a lower temperature. Furthermore, our results indicate that changes in the lower molecular-weight range (from 4900 to 10400) had a less pronounced effect on the LCST. This observation could arise from the structural evolution of HPGs from relatively open structures at low molecular weights to denser spheroid shapes at high molecular weights.

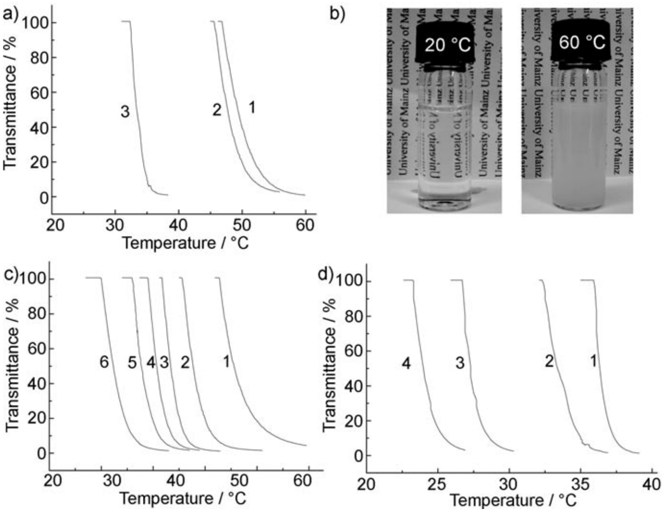

Figure 1. a) Effect of temperature on transmittance of 1) HPG_{18}-NIPAM, 2) HPG_{38}-NIPAM, and 3) HPG_{76}-NIPAM at pH 9.0 (2 mg mL^{-1}). b) Photos of solutions of HPG_{18}-NIPAM in water at 20.0 °C (left) and at 60.0 °C (right). c) Effect of temperature on transmittance of HPG_{38}-NIPAM (2 mg mL^{-1}) at 1) pH 8.0, 2) pH 9.0, 3) pH 10.0, 4) pH 11.0, 5) pH 12.0, and 6) pH 13.0. d) Effect of temperature on transmittance of HPG_{76}-NIPAM (2 mg mL^{-1}) at 1) pH 8.0, 2) pH 9.0, 3) pH 10.0, and 4) pH 11.0.

Since the HPG-NIPAM polymers possess tertiary amine groups in their structures, it can be expected that the pH of the solutions will affect the hydrophilic–hydrophobic balance in the polymers by changing their charge density. The results of turbidimetry measurements (Figure 1 c,d) revealed that the LCST of HPG-NIPAM polymers increased with decreasing solution pH. For example, the LCST of HPG_{38}-NIPAM shifted from 24.2 °C to 52.4 °C when the pH changed from 13.0 to 8.0. Moreover, at pH values below 7.0, all three HPG-NIPAM samples used in this study showed no phase transition in the experimental temperature range. Clearly, a decrease in the solution pH leads to protonation of the tertiary amine groups in the polymers. The corresponding increase in charge density enhances the overall hydrophilicity of the polymers, while the resulting charge repulsion causes expansion of the macromolecules, which in turn reduces the interaction of the NIPAM groups.[20] The trend of the pH dependence here should be a synergetic result of these two factors. Taken together, our experimental data suggest that the LCSTs of the HPG-NIPAM polymers can be rationally controlled both by structural parameters, such as molecular weight, and environmental factors, such as pH.

Monodisperse gold nanoparticles (Figure 2 a) with a diameter of 12.0 nm were prepared by the citrate-reduction method.[26] Zeta-potential measurements showed that the as-prepared nanoparticles were negatively charged ($\zeta = -38.0 \pm 0.6$ mV) owing to the stabilizing citrate ligands. Recently, Schneider and Decher demonstrated that such gold nanoparticles can be coated with positively charged polyelectrolytes through electrostatic interaction, thereby avoiding the formation of large aggregates.[27] Further developing this concept and relying on the polyfunctionalilty of the hyperbranched polymers, the positively charged HPG-NIPAM polymers (2.0 mg mL^{-1}) were coated on gold nanoparticle surfaces. The free polymers remaining in solution were removed by centrifugation at 12000 *g* for 25 min.[25] A less concentrated polymer solution (0.5 mg mL^{-1}) only led to nanoparticles with limited stability, which might be due to incomplete coating of the nanoparticles at this concentration. One direct piece of evidence of successful surface coating is that the surface charge of the nanoparticles ($\zeta = 17.4 \pm 0.8$ mV) was inverted after coating. As shown in Figure 2 b, most of the particles remained isolated on the TEM grid after coating with HPG_{76}-NIPAM, confirming that the coating process does not cause aggregation of the nanoparticles. The HPG-NIPAM-coated nanoparticles became highly stable in

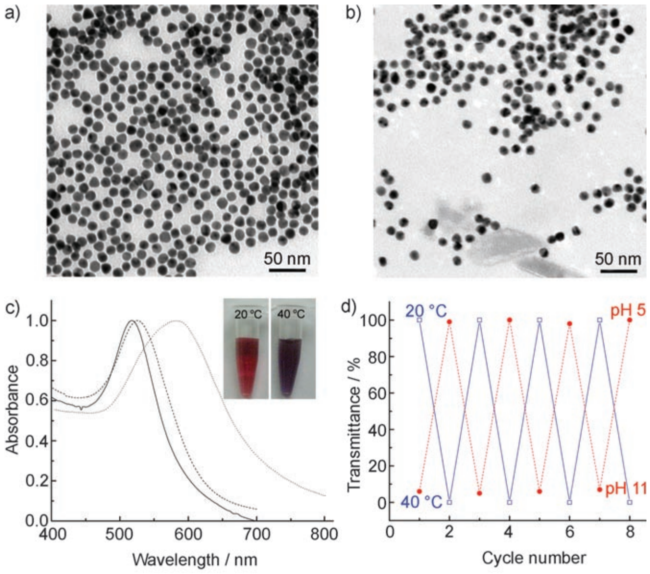

Figure 2. TEM micrographs of gold nanoparticles before (a) and after (b) coating with HPG_{76}-NIPAM. c) UV/Vis absorption spectra of the original gold nanoparticles (solid line), gold nanoparticles coated with HPG_{76}-NIPAM at 20.0 °C (dashed line), gold nanoparticles coated with HPG_{76}-NIPAM at 40.0 °C (dotted line); inset: photographs of aqueous solutions (pH 9.0) of HPG_{76}-NIPAM-coated gold nanoparticles at 20 °C and after heating up to 40.0 °C. d) Changes of transmittance during both pH variation cycles between pH 5 and 11 and heating–cooling cycles between 20.0 and 40.0 °C.

concentrated salt solutions (e.g., 1.0 M NaCl). In contrast, the original nanoparticles aggregated rapidly and eventually precipitated under the same conditions. Compared to the original nanoparticles, the surface plasmon band of the HPG_{76}-NIPAM-coated gold nanoparticles (at pH 9 and room temperature) was red-shifted by 9 nm (Figure 2c), which should be the result of an increase of the local refractive index change after coating with HPG_{76}-NIPAM polymers.[28]

Upon increasing of the temperature to 35 °C, the surface plasmon band of the coated gold nanoparticles became broader and further red-shifted by 50 nm with a concomitant change in solution color from clear red to opaque purple (Figure 2c), thus confirming that these gold nanoparticles are indeed thermosensitive. At 40 °C, the nanoparticles formed large aggregates of 925 nm in size, and their zeta potential dropped to 12.2 ± 2.5 mV, which most probably is due to the shielding effect caused by aggregation of the hydrophobic groups. Our results showed that the LCSTs of the polymer-encapsulated nanoparticles are slightly lower (3–9 °C) than that of the pure polymer. This finding could result from the significant reduction in conformational freedom of the HPG-NIPAM polymers after immobilization on the gold nanoparticle surfaces because binding on the nanoparticles imposes an additional boundary for the polymers to collapse, towards the nanoparticle core. Similarly, Li and co-workers found a decrease of 5 °C in the LCST for linear-PNIPAM-coated gold nanoparticles,[9] although there is a conformational difference between the globular hyperbranched polymers and the random-coiled linear polymers. On the other hand, this trend indicates that the nature of the linking bonds between thermosensitive polymer coatings and gold nanoparticles appears to play a less important role in this respect, considering that linear PNIPAM was attached to the nanoparticles through covalent Au–S bonds,[9] whereas the HPG-NIPAMs are coated through multiple electrostatic interactions. Similar to our adsorbed HPG-NIPAM, the LCSTs of the coated nanoparticles are also dependent on solution pH. For example, the HPG_{76}-NIPAM-encapsulated nanoparticles exhibit an LCST of 24.2 °C at pH 11.0, and 30.1 °C at pH 9.0, whereas at pH 5.0 the nanoparticles become completely soluble in the whole experimental temperature range. The pH-induced aggregation of the gold nanoparticles also leads to a distinct red-shift of the plasmon band. Figure 2d demonstrates that aggregation of these nanoparticles caused either by temperature or pH variation is completely reversible over multiple cycles, thus revealing the robust nature of this hyperbranched polymer coating.

The results of turbidity measurements of the HPG-NIPAM-coated gold nanoparticles are summarized in Figure 3. Clearly, by varying the molecular weight of the polymer and solution pH, the LCST of these nanoparticles can be readily controlled in the range of 25 to 55 °C. As depicted in Figure 4, these thermosensitive polyelectrolyte-coated gold nanoparticles possess potential for application as both temperature and pH nanosensors.

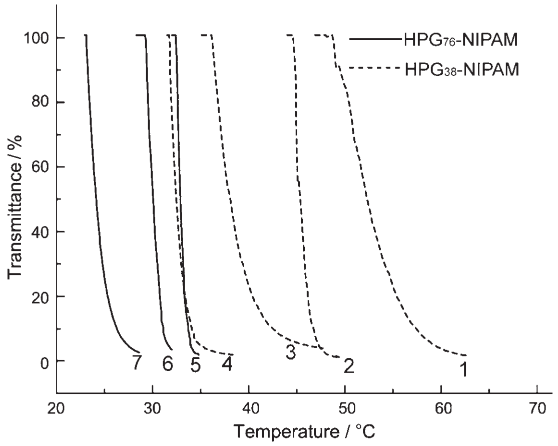

Figure 3. Effect of temperature on transmittance of Au nanoparticles coated with HPG_{38}-NIPAM (2 mg mL^{-1}) at 1) pH 8.0, 2) pH 8.5, 3) pH 9.0, 4) pH 10.0 and Au nanoparticles coated with HPG_{76}-NIPAM (2 mg mL^{-1}) at 5) pH 8.0, 6) pH 9.0, 7) pH 11.0.

In conclusion, we have developed a novel strategy to prepare stimuli-responsive gold nanoparticles by encapsulating the nanoparticles with polyvalent hyperbranched polyelectrolytes, leading to a sharp phase transition upon change of temperature or pH value. The unique globular structures of the hyperbranched macromolecules offer new possibilities to control phase-transition temperatures by variation of the molecular weight instead of changing the chemical nature of the polymer layer. In a more general context, this work

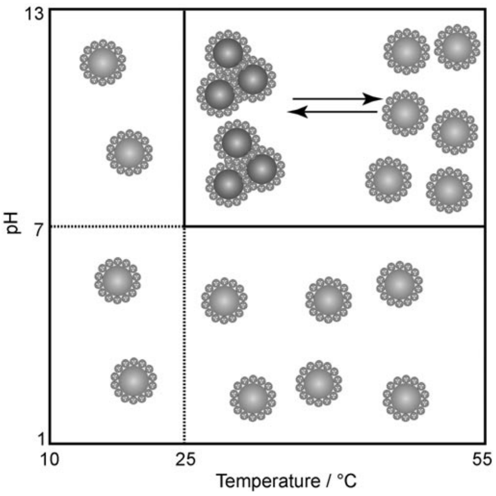

Figure 4. Illustration of effects of pH and temperature on dual-responsive Au nanoparticles coated with HPG-NIPAM.

demonstrates the intriguing potential of hyperbranched structures at interfaces.

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